REGIONAL AND LOCAL DRIVERS OF MASON BEE (GENUS *OSMIA*) DECLINE ACROSS THE EASTERN SEABOARD

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Though there has been much focus on honey bee (*Apis mellifera* L.) decline, wild bees, which are essential to both ecosystem functioning and crop pollination, are also facing declines across Europe and North America. Potential drivers of theses declines include competition with non-native species, landscape simplification, increased pesticide risk, and reduced diet diversity. Here, I ask 1. how an introduced wild bee impacts a closely related, native congener at a regional scale, 2. how landscape simplification, diet diversity, and pesticide risk interact to impact wild bee populations in apple agroecosystems, and 3. how pesticide risk levels compare between wild bees and honey bees in the same apple orchards during bloom.

To assess the impact of non-native *Osmia cornifrons* on the decline of native *O. lignaria* across the Eastern Seaboard, Ie used historical specimen records from 36 insect collections over 120 years. I found no evidence that *O. cornifrons* influenced *O. lignaria* decline; instead, their abundance (relative to other bees) has been decreasing since 1890, long before the 1977 introduction of *O. cornifrons*. Next, I was interested in exploring the drivers of *Osmia* performance in agroecosystems. Due to limited availability of *O. lignaria*, I assessed the response of nesting female *O. cornifrons* to landscape simplification, pesticide risk, and floral diet diversity in 17 NY apple orchards in 2015. In simplified landscapes, *O. cornifrons* produced fewer female offspring that weighed less, via reduced diet diversity and increased fungicide risk levels from Rosaceae (likely apple) pollen. Reductions in female offspring number and weight could lead to *O. cornifrons* population decline over time, as smaller-bodied bees

produce fewer offspring and have shorter life-spans, suggesting that further studies of wild bee declines should focus on landscape simplification, pesticide risk, and floral diet diversity as potential drivers. To assess whether the historic use of honey bees as models for wild bee decline is adequate, I directly compared one driver of bee decline, pesticide risk levels, in *O. cornifrons* and *A. mellifera* pollen in 14 apple orchards during bloom in 2015. For *O. cornifrons*, increasing apple land cover resulted in increased pesticide risk levels in their pollen provisions, via increased *Malu*s (crop) pollen collected. However, these relationships were not significant for honey bees, suggesting that their use as a model for all bee species may lead to inaccurate assessments of pesticide risk to some wild bee populations in agroecosystems.

My results show that *Osmia lignaria* decline is not necessarily exacerbated by *Osmia cornifrons* at the regional scale. At the local scale, I show that landscape simplification, increased pesticide risk, and reduced diet diversity could potentially lead to *O. cornifrons* population decline, via reduced offspring number and size. Finally, I show that it is essential to continue studying the drivers of wild bee decline, as honey bees do not provide an adequate model with which to assess health of all bee species. By continuing to research the underlying causes of wild bee decline at both the regional and local scales, we can better preserve these important pollinators.

BIOGRAPHICAL SKETCH

Mary Centrella has always been passionate about nature, a passion born in the big skies, vast plains, and beckoning mountains of wild Wyoming. At the University of Wyoming, from which she received a Bachelors of Arts in Spanish and a Bachelors of Science in Zoology in 2013, she was lucky to meet Dr. Scott Shaw and Dr. Michael Dillon, both of whom left big impressions on her future career by sparking her interest in wasps and bees. During her time as graduate student at Cornell University, Mary has created a project that helps to bridge the gap between multiple scientific fields. At Cornell, Mary discovered her true calling: sharing science with others through outreach and extension. She is poised for a career bridging the gap between scientific research and the community and will be starting as the Director of the Pesticide Management Education Program for New York State in the fall.

Through her research, Mary works to understand the drivers of mason bee decline, so that we can preserve the health and biodiversity of our important wild bee pollinators. During her undergraduate degree, Mary was a Goldwater Scholar, and as a graduate student, she received the National Science Foundation Graduate Research Fellowship. Mary is grateful for these awards, as they have helped her to achieve her academic and career goals. While at Cornell, Mary served as a Fellow for the Center for Teaching Excellence and was also a mentor for the LGBT Resource Center. She has enjoyed extension and outreach activities, such as building bee nests with the community at Judy's Day, and presenting her research to the Apple Research Development Program. One of her favorite moments was returning to the AMK Research Station in Grand Teton National Park, where she once worked as a maintenance woman, to speak about bee decline and biodiversity to over 150 park workers, community members, and scientists.

ACKNOWLEDGEMENTS

I am incredibly grateful to have had the opportunity to be a graduate student here at Cornell, in the Department of Entomology. The students, faculty, and staff have been instrumental in supporting me and I have come to regard many of them as close friends and talented collaborators. I am also grateful to the Ithaca community, which accepted me with open arms. I could not have achieved this work without the constant support of my friends and family, who are scattered all over the globe, and, of course, the patience and compassion of my amazing fiancée.

I feel very lucky to have had such a talented team of advisors. Thank you, Bryan, for your patience as you guided me to become a better writer and speaker. Thank you for all the opportunities you offered me, from allowing me to instruct a course with you in the tropics, to introducing me to leading researchers in our field at the Entomological Society of America. You are incredibly generous with your funding, your time, and your talent. You have cultivated a lab full of strong, warm, and smart scientists from whom I have benefitted immensely and with whom I will always be friends. You have taught me how to tell a story and your passion for wild bees has been a great motivator during my time in your lab.

Thank you Katja, for supporting me, not only in my thesis research, but also in my development as a scientist, and in my personal growth during these six transformative years. From assembling a team of people to help move hundreds of plants for my experiment, to orchestrating a lab-wide publication, your passion for science and your ability to motivate people has been inspiring. You have developed a collaborative, engaging lab full of gifted colleagues whom I will miss dearly. My project would have been impossible without your statistical prowess and expertise in experimental design. Most importantly, you have taught me how to be flexible when projects flounder, to “listen” to my data, and to stay optimistic and engaged in research.

I want to thank both Bryan and Katja for working so well as team. It was the combination of your talents and your ability to work well with each other that made this project, and truly my career, so successful.

This thesis is a tribute to the support and advice of my committee members, Jeff and Scott. Jeff, you hold everyone to a high scientific standard, but you also give them the tools to meet these expectations. I feel lucky to have had you as teacher and I have learned so much about toxicology from you. You inspired me to be passionate about pesticides, a subject I had minimal knowledge of before coming to New York. I have always appreciated your candid attitude, and the advice you give improves not just my research, but my understanding of science. Scott, I have enjoyed collaborating with you immensely. You have the unique ability to pull together ideas from many different fields and you are always willing to share with others. Your dedication to pairing excellent research with equally vigorous extension and dissemination of that knowledge really sets you apart.

I want to thank my entire committee. Jeff, Scott, Bryan, and Katja, you have helped me create a project that encompass a variety of fields of research and you have given me the tools and the confidence to pursue a career in scientific extension. I look forward to continuing to learn from all of you in the future.

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CHAPTER 1

RANGE EXPANSION OF NON-NATIVE *OSMIA CORNIFRONS* (HYMENOPTERA: MEGACHILIDAE) AS A RESPONSE TO, NOT A DRIVER OF, NATIVE *O. LIGNARIA* DECLINE

Abstract:

Wild bee decline is well-documented across Europe and North America. Recognized drivers of this decline include climate change, agricultural practices, pests, parasites and pathogens, and competition with non-native bee species, though the latter has been relatively understudied. Indeed, over the past 30 years, 32 bee species have been established outside of their native range. Non-native bees can negatively impact native bees via competition for resources, vectoring of foreign pathogens, and reproductive disruption. However, our study is the first to our knowledge to examine the impacts of a non-native bee on a closely-related congener in both a spatial and temporal context. Here, we use historical specimen data from 36 insect collections spanning 120 years to ask whether the recent introduction of mason bee *Osmia cornifrons* influenced the decline in native mason bee *O. lignaria*. We predicted that decline would be most pronounced after detection of *O. cornifrons* and where both species were in sympatry. We found that the region of maximum extent of *O. cornifrons* increased in area over time, and that *O. lignaria* decline is more pronounced in the region of overlap with *O. cornifrons*. However, *O. lignaria* abundance (relative to other bees) has decreased since 1890 along the Eastern Seaboard, long before the 1977 introduction of *O. cornifrons*, and there was no significant difference in the rate of decline before and after *O. cornifrons* detection, suggesting that *O. cornifrons* did not influence *O. lignaria* decline. Instead, our results indicate that *O. lignaria* decline is likely due to historical factors that differed between the region of allopatry and the region of sympatry of both

species. Our results emphasize the importance of analyzing historical population changes when studying the effects of non-native species on native bees, to be able to better understand the underlying mechanisms of wild bee decline.

Introduction:

Wild bees are important pollinators of flowering plants. Preserving their biodiversity is essential to preserving ecosystem diversity and functioning, as 85% of flowering plants depend on pollination (Ollerton, Winfree, & Tarrant, 2011). Unfortunately, analyses of historical insect collection records have shown clear evidence of wild bee decline across Europe and North America. Indeed, 9.2% of wild bee species in Europe are experiencing population declines (Nieto et al., 2014). Native bee richness has declined across 52% of Britain and 67% of the Netherlands (Biesmeijer, 2006). In Britain in particular, one third (33%) of wild pollinator species, including many wild bee species, have declined between 1980 and 2013 (Powney et al., 2019), with rapid wild bee extinctions beginning in the 1920's (Ollerton & Crockett, 2015). Of 187 native North America bee species, 53 of them (or 28%) have significantly declined over the last 140 years (Bartomeus et al., 2013). In just the last 20 years, four North American *Bombus* species have declined in relative abundance by 96% and their ranges have contracted between 23 and 87% (Cameron et al., 2011). Factors implicated in wild bee declines include: 1. climate change and environmental pollution, 2. agricultural practices (including bee management, land-use change, pesticide application, and changes in floral resource availability), 3. pests, parasites, and pathogens, and 4. introduction of non-native species (Goulson, Nicholls, Botías, & Rotheray, 2015; Potts et al., 2010; Vanbergen, 2013).

While the negative impacts of climate change, agricultural practices, and pests, parasites, and pathogens have been well-documented, we know much less about the impact of non-native bees on

native bee declines, despite the fact that bee introductions are widespread. World-wide, there are over 80 bee species that have been established outside of their historic, native ranges, 91% of which were introduced by human activity (Russo, 2016), while the introduction history of the other 9% remains unknown or due to natural range expansion. Eighty percent of bee introductions were accidental, while 20% were deliberately introduced, often for the purpose of agricultural pollination (Russo, 2016). While most studies focus on the impacts of introduced species in the family Apidae (30 species), there are and equivalent number of introduced species belonging to the family Megachilidae (33 species), likely because their stem-nesting habit facilitates accidental and intentional transport. Globally, 69% of non-native bees are stem-nesters. It is important that we understand the impacts of these bee species introductions, as they have become more common in recent years. In fact, over a third (32) of the documented non-native bees established in the last 30 years (Russo, 2016).

Some of the potential impacts that non-native bee species can have on their native congeners include: 1. competition for floral resources and nesting sites, 2. increased pathogen and parasite pressure, and 3. reproductive disruption via interspecific mating (Goulson, 2003; Russo, 2016; Stout & Morales, 2009). For instance, increasing abundance of non-native honey bees has been associated with reduced diet breadth and reduced per-trip pollen collection in native bumble bees (Stout & Morales, 2009; Thomson, 2004). Direct competition between bees on flowers has also been documented; increased floral visitation by non-native *Bombus* corresponded with decreased visitation of a native species (Morales, Arbetman, Cameron, & Aizen, 2013). In addition, pathogen and parasite pressure has been shown to increase as a result of introduced species, via direct transfer of pathogens associated with the introduced species to the native species (Alger, Burnham, Boncristiani, & Brody, 2019; Fürst, McMahon, Osborne, Paxton, & Brown, 2014; Graystock et al., 2013). Finally, interspecific mating with non-native *B. terrestris* led to egg inviability in 30% of native *B. hypocritica* queens (Kanbe, Okada, Yoneda, Goka, & Tsuchida, 2008), and genetic introgression between *A. mellifera* subspecies led to

genetic swamping of the native subspecies via gene flow(Jensen, Palmer, Boomsma, & Pedersen, 2005). These potentially devasting effects on native bee populations make it essential that we study the impacts of introduced bee species on their native congeners.

In this study, we ask whether a recently established, stem-nesting megachilid bee, *Osmia cornifrons*, is impacting the abundance of a similar native species, *O. lignaria,* along the Eastern Seaboard of North America. Like its native counterpart, *Osmia cornifrons* is a solitary, mass-provisioning, floral-generalist bee with one generation per season. It was intentionally introduced from Japan to North America via the USDA Beneficial Insect Introduction Laboratory at Beltsville, Maryland in 1977 for pollination purposes (Batra, 1978). Since its introduction, *O. cornifrons* relative abundance has increased exponentially through 2011 (Bartomeus et al., 2013). Anecdotal evidence also points to range expansion across the Eastern Seaboard of the US, with some isolated populations in the American west. While *O. lignaria* appears to have been in decline since the early 1900's (Bartomeus et al., 2013), it is unknown what impact *O. cornifrons* has had on *O. lignaria* populations since its introduction. However, there has been recent concern from insect collectors along the Eastern Seaboard who have noted that *O. cornifrons* appears to be more prevalent compared with *O. lignaria*.

There are numerous reasons why we might expect introduced *O. cornifrons* to negatively impact native *O. lignaria* populations. The two species have comparable foraging ranges and body size and they utilize nests of similar diameter (Guédot, Bosch, & Kemp, 2009; Kitamura & Maeta, 1969). Both species have a proclivity for pollinating tree fruits (Batra, 1995; Bosch & Kemp, 2001; Torchio, 1982), and they share a similar diet breadth (Haider, Dorn, Sedivy, & Müller, 2014). Thus, there is potential for competition for nesting and floral resources, especially when resources become scarce. In addition, it was recently shown that a Japanese fungal chalkbrood pathogen (genus *Ascosphaera*) was transferred from Japan by *O. cornifrons* (Hedtke, Blitzer, Montgomery, & Danforth, 2015), and there is concern this pathogen could infect *O. lignaria*.

Here we explore whether temporal and spatial overlap with *O. cornifrons* exacerbated *O. lignaria* decline in the eastern US, using data from 36 public and private insect collections spanning 120 years. We first established how abundance (relative to other bees) changes over time for both species, and we established the geographic range for *O. cornifrons*. Then, in order to address our question, we examine changes in *O. lignaria* abundance (relative to other bees) over time both **before and after** the detection of *O. cornifrons* in collection records, as well as changes in relative *O. lignaria* abundance over time in the regions of **allopatry and sympatry** (using the current established geographic range) with *O. cornifrons*. If *O. cornifrons* has had a negative impact on *O. lignaria*, the most significant decline in relative *O. lignaria* abundance should occur both **after** *O. cornifrons* was detected and **within the region of sympatry** with *O. cornifrons* (see Fig. 1.1). To our knowledge, this is the first study to examine the impacts of a non-native bee on a closely related native congener in both a spatial and temporal context. We predict the following:

1.Relative to all bees, *O. cornifrons* will increase in both abundance and range over time.

2.Relative to all bees, *O. lignaria* will decrease in abundance over time.

3. The decline in the relative (to all bee specimens) abundance of *O. lignaria* should be most pronounced **after** *O. cornifrons* detection and **within the region of sympatry** with *O. cornifrons* (see Fig. 1.1).

4. Relative to **both *Osmia* species**, *O. lignaria* abundance will decrease in prevalence **after** *O. cornifrons* detection and **within the region of sympatry**.

Methods:

Collection specimens:

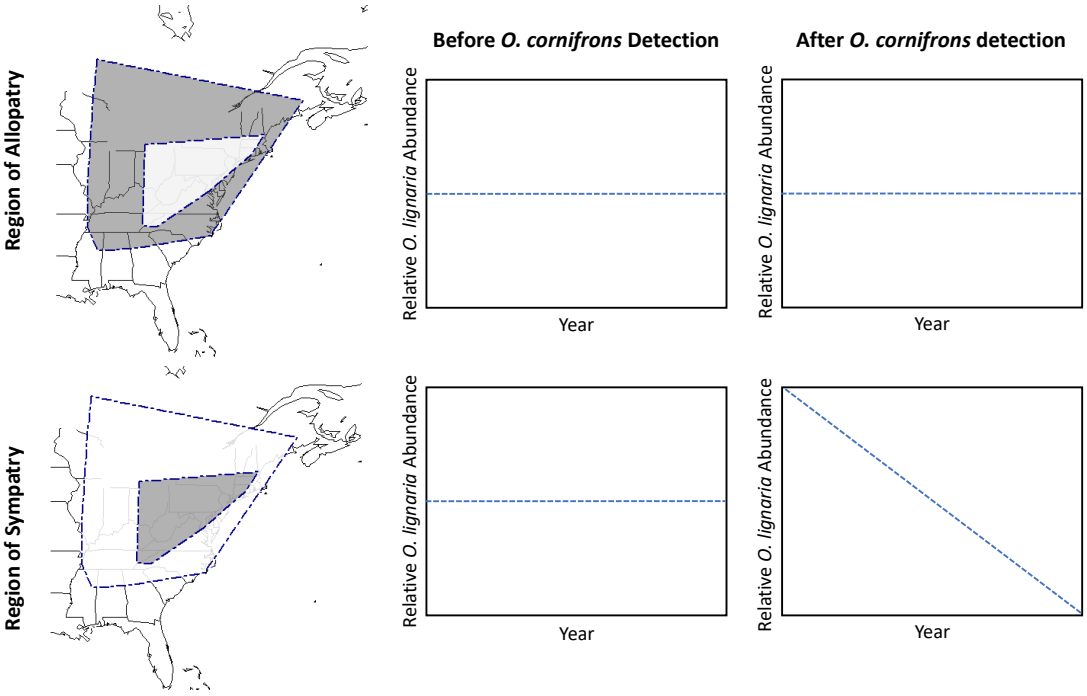

Figure 1.1. Graphical representation of prediction 3 (see Introduction): We predict that the relative decline in *O. lignaria* will be most pronounced where native *O. lignaria* overlaps with introduced *O. corniforns* in space and time. We expect that *O. lignaria* decline will be most pronounced both **after** *O. cornifrons* detection and also **inside the region of sympatry**, compared with before *O. cornifrons* detection inside the region of sympatry, before *O. cornifrons* detection in the region of allopatry, and after *O. cornifrons* detection in the region of allopatry.

In an effort to characterize the region of *O. cornifrons* establishment from the point of introduction in Beltsville, MD, we obtained specimen data for *O. cornifrons* and *O. lignaria* from 36 insect collections, located primarily along the Eastern Seaboard of the US. Of these collections, 16 were housed in the American Museum of Natural History Arthropod Easy Capture Specimen Database (https://research.amnh.org/pbi/locality/) and 18 were sourced from private and university collections (Table A1.2). Information was digital, except from 6 collections where curators sent us new specimens not yet digitally catalogued. The information from these 6 collections was uploaded to the AMNH database as part of this project. All collections, their locations, and their abbreviations are shown in Table A1.2. Data from the AMNH repository were downloaded in early 2017, while data from other repositories were solicited from fall of 2013 through fall of 2014.

Records collected outside the months of April, May, and June were discarded, as this is outside of typical mason bee activity, and usually indicated bees were lab-reared. To ensure accurate comparison of abundance across mason bee populations, only net-collected bees were used for our analysis, and records from trap-nested bees were excluded. Records lacking a year and month were also discarded for lack of information. We removed records without latitude and longitude or where the latitude and longitude did not match the locality listed. For records where only a locality was listed, latitude and longitude were added by choosing the center-most point in the locality listed. Records with localities larger than a 15 km radius (for instance, “New York City” or “Yellowstone National Park”) were removed to ensure accuracy of our specimen distributions. After refining our data set, we had 2,921 *O. lignaria* and 721 *O. cornifrons* specimens from 36 collections.

Mitigating Collection Bias:

We see four major problems to overcome when using museum collection data: 1. Collection effort is not exhaustive, 2. Collection effort is variable through time and space, 3. There is possibility of collection and/or collector bias, and 4. Collection motivation, or whether collectors are interested in single vouchers versus a series of specimens from one locality, is unpredictable (Bartomeus et al., 2013; Boakes et al., 2010; Ponder, Carter, Flemons, & Chapman, 2001; Bartomeus et al., 2018).

In order to account for the first three problems, past researchers have compared the abundance of focal taxa with the abundance of background taxa (Ponder et al., 2001), so that focal species abundance is measure *relative* to background collection effort. As long as focal taxa are collected with the same overall effort as the background taxa, then random variation in collection effort through space and time or biases towards certain collections or collectors should be accounted for in the background taxa. Because we assume that collectors or collections interested in bee specimens are likely to collect all bees at fairly similar rates, we chose to use all bee specimens collected as our background taxa, hereafter referred to as "background bees". Although collection efforts will never be exhaustive, if we see fewer *O. lignaria* specimens over time while the background bee abundance stays stable, we can be more confident that decline in the focal species is reflective of a true reduction in their population abundance. Because it was not feasible to procure background bee data across all 36 collections, we obtained background bee data from the top three repositories (AMNH, USGS, and UTAH; see Table A1.2) containing the most *O. cornifrons* (87%) and *O. lignaria* (45%) specimens (Fig. A1.1, Table A1.1). For all analysis of *Osmia* abundance relative to all bees over time (part of prediction 1, predictions 2 and 3), we limited analysis to specimens from just these 3 repositories (18 collections, as 16 collections were housed under the AMNH), while analysis of *O. cornifrons* range and of *O. lignaria* abundance relative to both *Osmia* species (part of prediction 1, prediction 4) used data from the 36 collections (see justification for this decision in paragraph after next). The use of 18 collections (3 repositories) instead of 36 for abundance analyses reduced the number of *O. lignaria* specimens from 2921 to 1324 and the

number of *O. cornifrons* from 721 specimens to 627 (Table A1.1). The background bee data were refined as described above for the *Osmia* data except that, due to the considerable size of the background dataset (260,8747 specimens), we were unable to check the accuracy of latitude and longitude against locality listed. Only specimens with both genus and species names were kept for analysis. Family names were added where absent and misspellings corrected (according to Michener, 2007). After refining our data set, we had 257,105 total background bee specimens (Table A1.1).

In order to minimize bias due to collection motivation (problem 4 above), we analyzed only unique collection events, or "UCEs". The UCEs comprised collection events that had a unique species, collector, date, and locality (see Bartomeus et al., 2013). Thus, if collectors were collecting a long series of the same species at the same locality, they would not erroneously bias our analysis. While this process reduced our background data by almost 75% and reduced our *Osmia* data by 50% (see Table A1.1 for), it was essential to reduce bias.

Though UCEs were used to address all predictions, it was not necessary to use background data for the analysis of *O. cornifrons* range area, and the analysis of *O. lignaria* abundance relative to the both *Osmia* species. For range area, we were not interested in specimen density, rather in their latitude and longitude, which we had checked for accuracy (see above). Even though our range area calculations were unlikely to capture the true extent of *O. cornifrons* populations, we were confident of the localities of the specimens available to us. When comparing *Osmia* species to each other, collection effort, variability, and bias were likely similar for both species, because most collectors do not target one bee species above another in the field, as it is difficult to identify bees on the wing or in the net. Thus, for *O. cornifrons* range and *O. lignaria* abundance relative to the abundance of both *Osmia* species (part of prediction 1, prediction 4), we used UCEs from all 36 collections, while our analysis of relative abundance (part of prediction 1, predictions 2 and 3) was limited to the 18 collections with background data.

Even after refining our data set data and the UCE calculation, our sample size is substantially larger than previous assessment for both species; we have 1,732 *O. lignaria* UCEs compared with 130 previously assessed and 407 *O. cornifrons* UCEs compared with 32 (Bartomeus et al., 2013). Even for relative abundance assessments with specimens from only 18 collections, the number of *O. lignaria* and *O. cornifrons* UCEs are still higher (1014 and 367, respectively) than previously assessed.

Spatial and Temporal Limitations:

Because 2013 was the final year with current data for some of our collections, we cut off analysis at this year. Indeed, data collection for both species is much lower after this year (Fig 2A). Another reason to limit our study to 2013 is to avoid bias from a time-lag between bee collection and documentation in the newer (2014) collection records. Although the first *O. lignaria* record was from 1824, *O. lignaria* UCE incidence was low between that time and 1889 (Fig. 1.2A, Fig. A1.1B). Indeed, 1890 was the first year where the background data were represented by as many as 25 UCEs and where *O. lignaria* had over 5 UCEs from more than a single collection (Fig. 1.2A). Including these early years would incorrectly weight our analysis at zero UCEs for *O. lignaria*. These zeros do not reflect population abundance, rather they correspond with low collection effort, or collection effort at fewer collections (see Fig. A1.1). For this reason, we used 1890 as the starting point for our analysis.

Because the Eastern Seaboard was the primary region of invasion and most of our collections were located within this region (Table A1.2), we focused our analysis east of the Mississippi, or East of -90 DD longitude (Fig. 1.1). We expected this region to fully encompass the range of *O. cornifrons* up to 2014, based on communications with many of the collectors and curators in this study. While some *O. cornifrons* are now found near Utah and Oregon (Dave Hunter & Theresa Pitts-Singer, pers. comm.), these western populations are likely due to human-mediated dispersal. Perhaps the introduction of *O. cornifrons* to Utah in 1965 that was deemed unsuccessful could have resulted in undetected populations

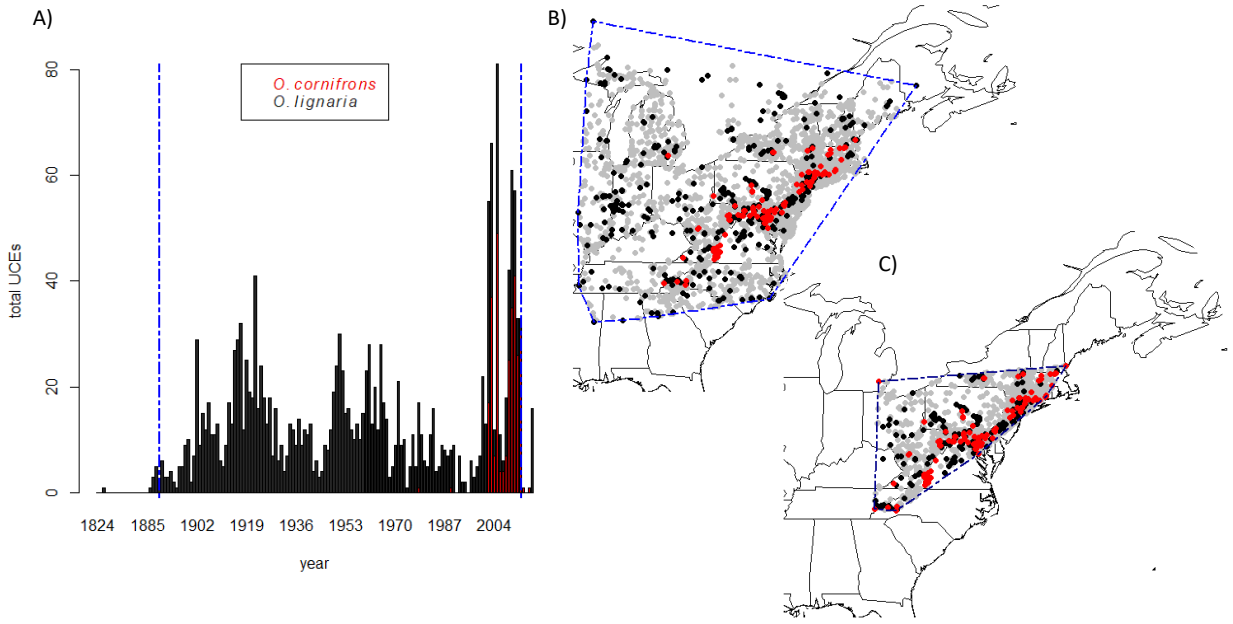

Figure 1.2. The summed unique collecting events (UCEs) across all 36 collections per year from our first *O. lignaria* specimen in 1824 through 2017 for both species, with *O. lignaria* bars in black and *O. cornifrons* bars in red (A). Dashed blue lines show the time period used for analysis: 1890-2013. The polygon including all *O. lignaria* UCEs (B) and the polygon containing all *O. cornifrons* UCEs (C), or the region of sympatry for our study. The region of allopatry was considered the part of total region (B) that did not overlap with the region of sympatry (C). Red points indicate *O. cornifrons*, black points *O. lignaria*, and grey points background bee UCEs, and dashed blue lines represent the perimeters of the regions/polygons (B, C).

(Batra, 1978), or perhaps bees were inadvertently transported west in recent years, due to the burgeoning commercial management of *Osmia* pollination services. Our -90 DD spatial limitation also ensured that our study only included one subspecies of *O. lignaria*, *O. lignaria lignaria*, as it is possible there might be differences between subspecies.

Establishing O. cornifrons detection and regions of allopatry and sympatry:

In order to assess the influence of *O. cornifrons* on *O. lignaria* decline, we established a time of detection for *O. cornifrons* and a region of sympatry and allopatry for both species, so that we could analyze relative *O. lignaria* abundance inside and outside sympatry and before and after *O. cornifrons* detection.

Although *O. cornifrons* was introduced in 1977, there was a delay until specimens appeared in the collection records in detectable amounts. Between 1977 this time and 2002, only 3 UCEs were collected (1978, 1989, and 2001). In 2002, UCE numbers increased dramatically and 26 UCEs were collected in that year (Fig. 1.2A). There is likely a lag between actual *O. cornifrons* incidence and their appearance in the collection records, but we would expect to also see a similar lag in *O. lignaria* in the collections. For this reason, we identified 2002 as the first year of detection for *O. cornifrons* in the collections, as this was the first time that they were found in substantial enough numbers to likely influence *O. lignaria*. For analysis, the years considered "**before"** *O. cornifrons* detection are 1890 to 2001, and years considered "**after"** *O. cornifrons* detection are 2002 to 2013. We assumed that, before detection in collections, *O. cornifrons* would be unlikely to influence *O. lignaria* in the records; in other words, we assumed that the lag time in collection response for both species would be similar. After our temporal and spatial limitations, there were 1448 *O. lignaria* UCEs before *O. cornifrons* detection and 284 after detection, and 362 *O. cornifrons* UCEs after their 2002 detection (Table A1.1).

We used data from all 36 collections (Table A1.2) to establish a geographic region of maximum extent with our study bounds (east of -90 DD in North America), for both *O. cornifrons* and *O. lignaria.* These regions encompassed all localities where specimens were collected from first detection through 2013. We used the computer program R (version 10.6.1) to created two convex-hull polygons (Fig. 1.2B&C) around the perimeter of both species UCEs using the owin and bbox functions in the spatstat package (Baddeley & Rubak, 2015). Polygon vertices can be found in Appendix 1 (Fig A1.4). The polygon encompassing *O. lignaria*, was 2,417,049 km^2, including 1,104 *O. lignaria*, and 52,817 background bee UCEs. The polygon encompassing *O. cornifrons* was 535,289 km^2, including 402 *O. cornifrons*, 661 *O. lignaria*, and 38,632 background bee UCEs (see Table A1.1). The polygon encompassing the maximum geographic range of *O. cornifrons* was entirely enveloped by the *O. lignaria* polygon, so that the *O. cornifrons* polygon was considered the "**region of sympatry**", while the perimeters of the *O. lignaria* polygon surrounding the region of sympatry were considered the "**the region of allopatry**" (see graphics in Fig. 1.1, Fig. 1.2).

Statistical Analysis of Predictions:

All statistical analysis was conducted using linear regression. Unless otherwise stated, response variables were estimated by single-predictor models with year as the explanatory variable. Models were created using the lm function in the lme4 package (Bates, Mächler, Bolker, & Walker, 2015) in the computer program R (version 3.5.1).

To quantify the relative abundance of each species to the background bees, we used the following equation:

$$Osmia\ sp.\ \text{Abundance (relative to all bees)} = \Sigma \text{ per year} \frac{Osmia\ sp.\ \text{UCEs}}{\text{Background Bee UCEs} + Osmia\ sp.\ \text{UCEs}}$$

In order to measure the changes in relative *O. lignaria* and *O. cornifrons* abundance over time, we created models using the above metric as our response variable and year as the predictor. In order to

measure the change in *O. cornifrons* range over time, we took the area of the convex hull polygon that encompassed all *O. cornifrons* UCEs per year and used this as our response variable with year as the predictor (Fig. 1.3). To compare the slopes of the relative *O. lignaria* abundance over time pre and post detection of *O. cornifrons,* we created a linear model with the sum *O. lignaria* UCEs per background bee UCEs as the response and the multiplicative interaction of year and time-frame (before and after *O. cornifrons* detection) as the predictor. We then compared the slope of this model before and after *O. cornifrons* detection using least squares means with the lstrends function in the lsmeans package (Lenth, 2018) in R. To quantify *O. lignaria* abundance relative to both *Osmia* species we used the following equation:

$$O.lignaria \text{ Abundance (relative to both } Osmia\ spp.) = \Sigma \text{ per year} \frac{O.lignaria \text{ UCEs}}{O.cornifrons \text{ UCEs} + O.lignaria \text{ UCEs}}$$

In order to measure *O. lignaria* abundance relative to both *Osmia* species over time, we created a linear model with the above variable as our response and year as the predictor. Because data were proportional, all model responses were log transformed. Models were visually inspected for homoscedasticity and linearity. The model characterizing relative *O. lignaria* abundance in allopatry after *O. cornifrons* detection did not meet the normality criteria, so the response was cube transformed. We created geographical maps, conducted spatial analysis of UCEs, and constructed polygons using the spatstat package in R (Baddeley & Rubak, 2015). Plots were created using the ggplot2 package in R (Wickham, 2016).

We assessed the change in background bee abundance both before and after *O. cornifrons* detection and in the regions of allopatry and sympatry with year as the predictor (Fig. A1.2). We also assessed the contribution of each of the top three repositories and the private collections to the abundance of each species over time by summing the number of UCEs collected per species per year

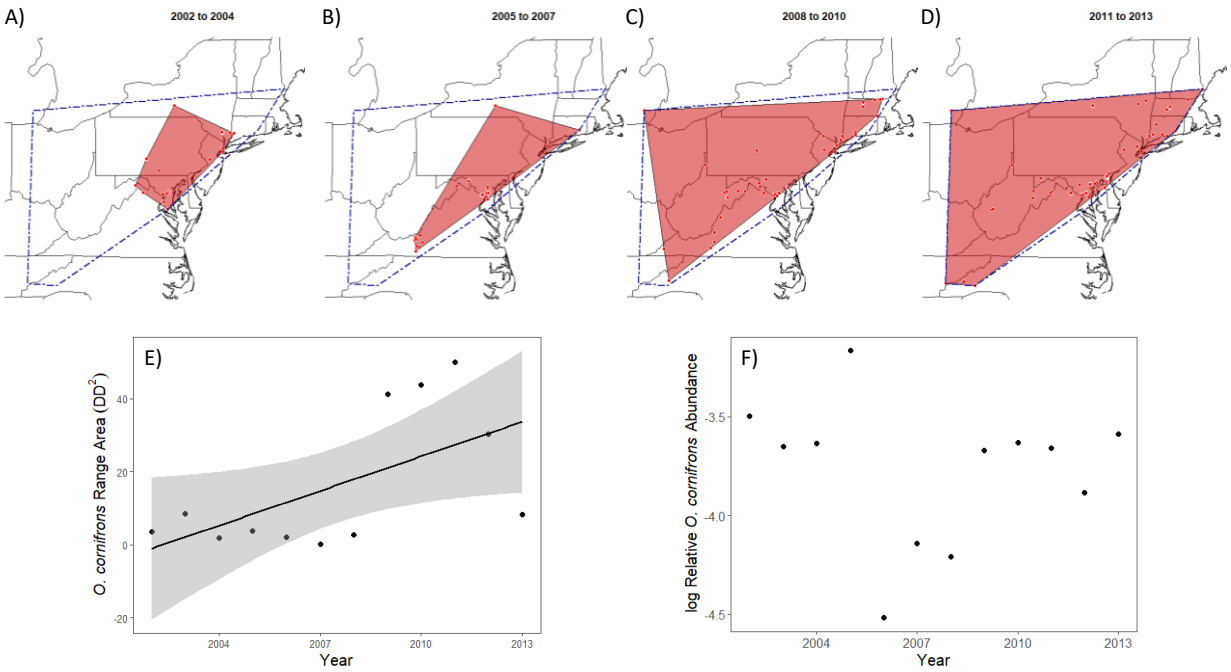

Figure 1.3. The spatial range of *O. cornifrons* UCEs (red polygon) from 36 collections from 2002 through 2013 in 4 consecutive time steps (A-D). There was a significant increase in relative *O. cornifrons* range area over time (E), and no change in relative *O. cornifrons* abundance over time (F). Points represent the area (in square decimal degrees) of the polygon containing all *O. cornifrons* UCEs per year from 36 collections (E). Points represent the yearly sum of *O. cornifrons* UCEs per background bee UCEs from 18 collections (F). Smooth line shows a significant relationship, and grey shading about the line shows the 95% confidence interval.

from each collection group (see Fig. A1.1). Boxplots were created with base R. The results for these assessments can be found in Fig. A1.1 and Fig. A1.2 in Appendix 1.

Assessment of Land Cover, Temperature, and Precipitation:

In response to our results, we compared mean temperature and summed precipitation from the PRISM climate group database inside and outside the region of *O. cornifrons* detection (Northwest Alliance for Computation Science and Engineering, 2019; Table A1.3)Temperature and precipitation were calculated for the month of April only, when the most *Osmia* specimens were collected. Early temperature and precipitation estimates were averaged across years every ten years starting at the earliest available year (1895) through 1995. More recent observations were averaged across three-year intervals starting the year before *O. cornifrons* detection, in 2001, through the extent of our study in 2013. For both estimates, portions of the region of allopatry that spanned into Canada were excluded from the analysis, due to low UCE prevalence there and because of a lack of and comparable climate information.

Results:

As expected, we found a significant increase in the *O. cornifrons* range area over time (R^2=0.291, $F_{1,10=}$5.52, p=0.041; Fig. 1.3E), but their expanding range did not correspond with increased relative abundance over time (R^2=-0.087, $F_{1,10}$=0.118, p=0.739; Fig 1.3F), likely because background bee abundance was also increasing, albeit only marginally, during this time (R^2=0.181, F1,10=3.435, p=0.095; Fig. A1.2). In accordance with our predictions, there was a significant decline in relative *O. lignaria* abundance across the Eastern Seaboard from 1890 through 2013 (R^2=0.089, $F_{1,122}$=13.02, p<0.001; Fig. 1.4).

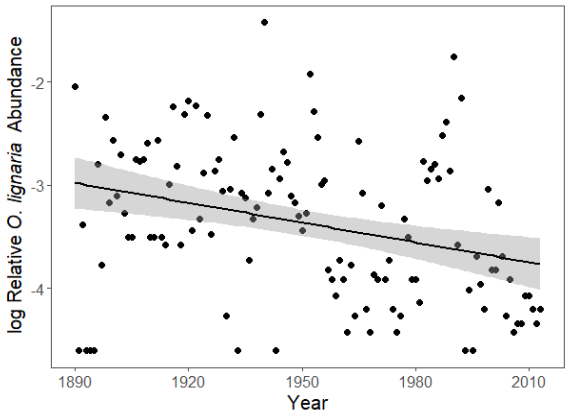

Figure 1.4. There was a significant decrease in the log of the relative *O. lignaria* abundance across the Eastern Seaboard (see *O. lignaria* polygon Table A1.4) from 1890 through 2013. Points represent the yearly sum *O. lignaria* UCEs per background bee UCEs from 18 collections, smooth line shows a significant relationship, and grey shading about the line shows the 95% confidence interval.

We had predicted that the decline in relative *O. lignaria* abundance would be more pronounced in the region of sympatry after detection of *O. cornifrons* (see Fig. 1.1). Indeed, in allopatry, *O. lignaria* did not decline. Instead, relative *O. lignaria* abundance actually *increased* in allopatry before *O. cornifrons* detection (R^2=0.046, $F_{1,110}$=6.319, p=0.013, Fig. 1.5) and did not change in allopatry after *O. cornifrons* detection (R^2=-0.022, $F_{1,10}$=0.763, p=0.403, Fig. 1.5). As we predicted, relative *O. lignaria* abundance significantly declined in the region of sympatry after detection (R^2=0.277, $F_{1,10}$=5.209, p=0.047, Fig. 1.5). Also consistent with our predictions, in sympatry after *O. cornifrons* detection, there was a decline in *O. lignaria* abundance relative to both *Osmia* species (R^2=0.356, $F_{1,10}$=7.075, p=0.024; Fig. 1.6). However, contrary to our predictions, *O. lignaria* also declined in the region of sympatry starting in 1890, long before *O. cornifrons* detection (R^2=0.086, $F_{1,110}$=11.4, p=0.001, Fig. 1.5). In addition, the rates of decline (slopes), before and after *O. cornifrons* detection in the region of sympatry did not differ (t=-0.761, se=0.069, df=120, p=0.448), suggesting the arrival of *O. cornifrons* did not exacerbate *O. lignaria* decline as we had predicted it would. The mean precipitation and temperature calculated inside the region of sympatry were not significantly higher than those quite values calculated in the region of allopatry; both the earlier, between 1895 and1995 across 6, three-year time periods, as well as later, between 2001 to 2013, across 11 ten-year periods (see Table A1.3).

Discussion:

To our knowledge, our results are the first to demonstrate a range area increase over time for *O. cornifrons.* Interestingly, the increase in range did not correspond to increased relative *O. cornifrons* abundance, likely because background bee abundance also marginally increased during this time (Fig S2). Thus, *O. cornifrons* populations were likely more numerous during this time, but they were not increasing in number faster than background bee collection. As found previously (Bartomeus et al.,

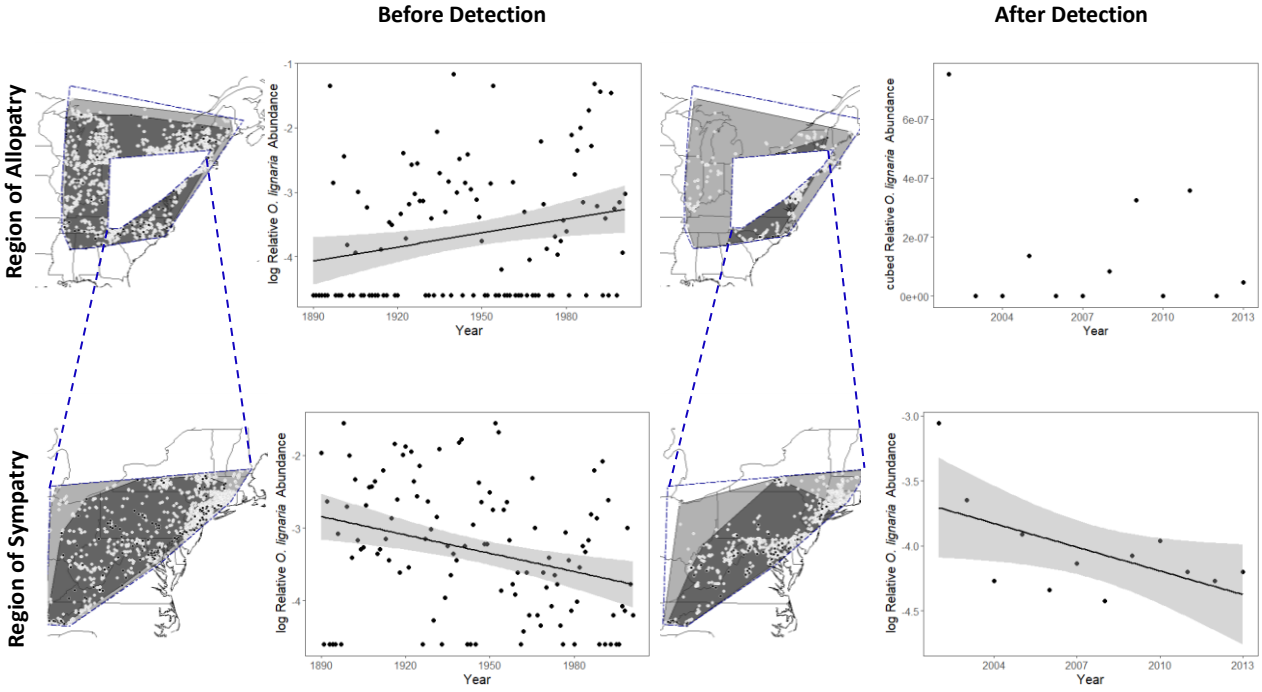

Figure 1.5. Relative *O. lignaria* abundance over time before and after *O. cornifrons* was detected in the region of sympatry and the region of allopatry with *O. cornifrons*. Points represent the yearly summed *O. lignaria* UCEs per background bee UCEs from 18 collections, smooth lines show significant relationships, and grey shading about the lines show the 95% confidence interval. Geographic figures show the range (black polygons) of *O. lignaria* UCEs (black dots) from 18 collections compared with the range (grey polygons) of background bee UCEs (grey dots) in each of the 4 scenarios. Blue dotted lines delineate the allopatric (upper figures) and sympatric (lower figures) regions.

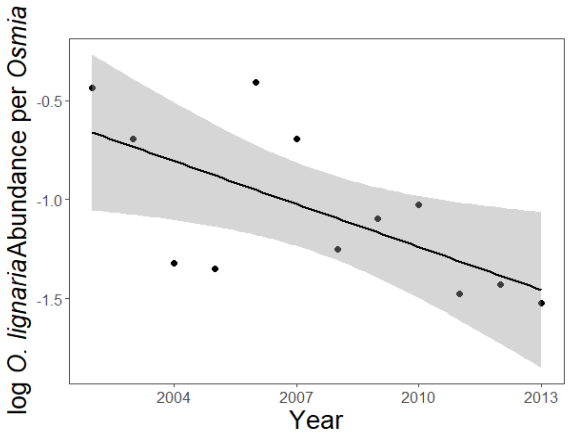

Figure 1.6. There was a significant decline in the log of the abundance of *O. lignaria* compared with the summation of both *O. lignaria* and *O. corniforns* abundance after *O. cornifrons* detection and in the region of sympatry. Points represent the proportion *O. lignaria* UCEs per both species UCEs per year based on data gathered from 36 collections. The line shows a significant relationship, and the grey shading about the line shows the 95% confidence interval.

2013), our results demonstrated an overall decline in relative *O. lignaria* abundance across the Eastern Seaboard from 1890 to 2013. Our results also confirm the pattern that collectors noticed: where both species overlap in space and time, *O. cornifrons* specimens seem to be "replacing" *O. lignaria* specimens. As we expected, *O. lignaria* declined inside of the region of sympatry, but did not decline outside of this region. However, *O. lignaria* were in decline since 1890 in this region, both before and after *O. cornifrons* detection, and the slope of decline was not different between the time-frames, suggesting that *O. cornifrons* did not influence *O. lignaria* decline. Instead, our results indicate that *O. lignaria* decline is likely due to historical factors that differed between the region of allopatry and region of sympatry.

Excluding non-native species, other potential drivers of wild bee decline include climate change, pesticide use, environmental pollution, pathogens, and land-use change (see Introduction; Goulson et al., 2015; Potts et al., 2010; Vanbergen, 2013). While they may have exacerbated declines, it seems unlikely that more recent environmental stressors such as climate change or pesticide use were the sole drivers of the observed, historical decline in *O. lignaria* occurrence. Indeed, global precipitation and temperature were relatively stable through the early 1900's (IPCC, 2013) and synthetic pesticide production did not begin in the US until the 1940's. In addition, average temperature and precipitation from 1895 through 2013 were quite similar between the two regions (see Table A1.3). Environmental pollution is a possible driver of decline, as the second US industrial revolution took place around the time we saw decline, from 1870 through 1914, and environmental pollution can impact offspring mortality in wild bees (Moroń et al., 2012). It is also possible that pathogen spill-over from honey bees to wild bees (see Fürst et al., 2014; Graystock et al., 2015) could have increased around this time. The Langstroth hive, invented in 1852, greatly facilitated management and movement of honey bee populations across the US, which could have increased the likelihood of pathogen vectoring to mason bees. However, it seems unlikely that industrial activity and migratory bee-keeping differed between the

two regions, making them less likely to be influencing *O. lignaria* decline, which was region specific. Also during the 20th century, there have been substantial changes in land cover across the Eastern US, such as an overall decrease in forested land and an increase in cropland area (Sleeter et al., 2013; Steyaert & Knox, 2008). While there is evidence that *Osmia* can rely on floral resources associated with forests and forest edges (Kraemer & Favi, 2005), as well as evidence that chemical inputs to agriculture can adversely impact bee populations (see Goulson et al., 2015), further research is necessary to verify whether these habitat types impact *O. lignaria* differently than *O. cornifrons* and also whether land-use differed significantly between the region of allopatry and the region of sympatry.

It is curious that *O. lignaria* decline has been most pronounced in the same region where *O. cornifrons* recently expanded its range. Indeed, though we predicted that the non-native species would drive abundance changes in the native species, our results suggest that, instead, *O. cornifrons* might be responding to *O. lignaria* declines in the region of sympatry. The fact that the range of *O. cornifrons* expanded into the same region where *O. lignaria* populations were already in decline suggests that the non-native species could be capitalizing on a niche that the endemic species had already begun to abandon. Indeed, *O. cornifrons* range expansion only occurred in the region where *O. lignaria* was in decline, and did not expand into the region of allopatry, where relative *O. lignaria* abundance was in fact *increasing.* This result further corroborates the idea that *O. cornifrons* were moving into an empty niche. In fact, non-native species are often implicated in native species decline, even though their presence may just be coincidental (Gurevitch & Padilla, 2004). For example, native cichlid decline in Lake Victoria in the 1960's was thought to be driven by the introduction of the Nile perch, but cichlid decline may have started 40 years earlier (in the 1920's) as a result of erosion (Verschuren et al., 2002). In addition, zebra mussels, introduced to the US in the 1980's, were thought to be the major reason for wide-spread decline in native bivalves (Williams, Warren, Cummings, Harris, & Neves, 1993), but it was later shown that native bivalve decline began much earlier, likely due to human harvesting, habitat loss, and

eutrophication (Neves, 1999). Another study found that non-native grasses were not the drivers of environment change, but rather the passengers; they were more dominant than native species because they were better able to adapt to long-term fire suppression, not because of direct competition (MacDougall & Turkington, 2005). It is even possible that the newly established *O. cornifrons* are *avoiding* the regions where *O. lignaria* is abundant. In fact, the non-native species could be occupying the niche where their native conspecific is in decline in order to avoid competition with them, opposite to what we had predicted.

Still, it seems unclear what factors are allowing *O. cornifrons* populations to remain stable in a region where their biologically similar, native relatives have been declining. One possibility could be trait differences between the two species. According to a study documenting wild bee decline in collection records across North America, bee species with narrower phenological windows of activity, larger body sizes, and smaller diet breadths were more likely to be in decline (Bartomeus et al., 2013). Although *O. lignaria* and *O. cornifrons* share similar phenological windows, *O. lignaria* females are on average 33.5 mg in dry weight, compared 22.2 mg dry weight for *O. cornifrons* (Guédot et al., 2009). A diet breadth assessment based on 28 and 50 pollen loads from *O. cornifrons* and *O. lignaria*, respectively, showed that both species collected 9 plant families, but their preferences were distinct: the top 3 families collected by *O. cornifrons* were Fabaceae, Rosaceae, and Brassicaceae, while the top three collected by *O. lignaria* were Salicaceae, Boraginaceae, and Fabaceae (Haider et al., 2014). These differences in floral diet collection between the two species could have driven the difference in their relative abundance over time, especially if certain resources were more prevalent inside the region of sympatry compared with outside this region; indeed, analysis of historical collections showed that wild bee declines coincide with pollen host plant losses (Biesmeijer, 2006). It is also possible that regional differences in land use could have impacted *O. lignaria* populations more negatively than *O. cornifrons* populations. For instance, for *O. cornifrons*, reduced forest land cover surrounding Japanese apple orchards has been

shown to lead to fewer provision masses produced per nest (Nagamitsu et al., 2017). Although *O. lignaria* have been shown to collect some forest/forest-edge pollens, they also appear to rely on shrubland resources, such as willow, boxelder, redbud, and blackberry in their pollen provisions (Kraemer & Favi, 2005). Thus, if these habitats differed between the two regions, we might expect to see differences in the relative abundance of these two species.

Conclusion:

Many studies of the impacts of non-native bees on native species focus on floral resource competition and pathogen and parasite transmission. However, by studying behavioral and biological changes without first ensuring there is a negative impact on native bee abundance at the population level, we may be placing the cart before the horse, resulting in overly negative predictions of the impacts of non-native species. Our results underline the importance of analyzing broad-scale, historical population changes when studying the effects of non-native species. Without detailed analysis of historical *O. lignaria* populations across both space and time, we may have incorrectly attributed more recent decline in *O. lignaria* to competition with *O. cornifrons*, when, in fact, decline appears to be spatially dependent and to have originated long before *O. cornifrons* was introduced. In order to help support *O. lignaria* populations, we should focus future efforts on pin-pointing the factor or combination of factors governing their historical decline, such as land-use changes or change in floral resource availability, rather than attempting to mitigate interactions with *O. cornifrons*, whose presence does not appear to influence their abundance. Future studies should focus on establishing the underlying causes of historical wild bee declines across multiple wild bee species.

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CHAPTER 2

LANDSCAPE SIMPLIFICATION REDUCES SOLITARY BEE OFFSPRING NUMBER AND WEIGHT IN AGROECOSYSTEMS VIA INCREASED FUNGICIDE RISK AND REDUCED FLORAL DIET DIVERSITY

Abstract:

Threats to bee pollinators such as landscape simplification, high pesticide risk, and reduced floral diet diversity are usually assessed independently, even though they often co-occur to impact bees in agroecosystems. Here, we measured pollen floral diet diversity, pollen pesticide risk, and bee response (as female offspring number and weight) in populations of the mason bee, *Osmia cornifrons,* at 17 NY apple orchards varying in landscape complexity. We tested the direct and indirect effects of landscape simplification, diet diversity and pesticide risk on bee response using path analyses. Our results showed fewer female offspring produced in simplified landscapes, via decreased floral diet diversity. In simplified landscapes, bees collected pollen with increased fungicide risk and increased proportion Rosaceae. Higher fungicide risk was marginally correlated with smaller female offspring, while increased proportion Rosaceae was indirectly associated with reduced female offspring weight, due to its positive, marginal, correlation with higher fungicide risk. We also found that landscape complexity, in the form of open areas, had a direct positive correlation with female offspring weight. To promote healthy *O. cornifrons* populations in apple, we must maintain floral resource diversity and landscape complexity, while reducing fungicide risk. More broadly, our results show that landscape simplification can negatively impact bee populations in agroecosystems indirectly through multiple, simultaneous threats. We must strive to understand the complexity of these simultaneous threats in order to maintain healthy populations of bees in the ecosystems where we rely on them most for pollination.

Introduction

Bees are essential crop pollinators, but there is rising concern that environmental threats that negatively impact bee health could lead to loss of pollination services in agroecosystems (Goulson, Nicholls, Botías, & Rotheray, 2015; Potts et al., 2010; Vanbergen, 2013). A major threat to bee populations is landscape simplification, or the conversion of natural areas to agricultural or urban landscapes. Landscape simplification can negatively impact bees at the community level, by reducing bee species richness and abundance (Connelly, Poveda, & Loeb, 2015; Mallinger, Gibbs, & Gratton, 2016; Winfree, Aguilar, Vázquez, Lebuhn, & Aizen, 2009). Landscape simplification can also impact bees at the population level, through reduced reproduction, survival, and body size (Renauld, Hutchinson, Loeb, Poveda, & Connelly, 2016; Williams & Kremen, 2007). Reduced bee abundance is likely to impact pollination, while reduced intraspecific body size has been shown to negatively affect yield (Jauker, Speckmann, & Wolters, 2016). Therefore, in order to maintain stable pollination services, it is imperative that we understand the mechanisms through which landscape simplification is impacting bees.

Landscape simplification can affect bees directly by reducing nesting resources (Threlfall et al., 2015) and increasing female foraging trip time (Jha & Kremen, 2013; Westphal, Steffan-Dewenter, & Tscharntke, 2006), which leaves nests vulnerable to predation and parasitism (Goodell, 2003). There is also compelling evidence that landscape simplification can affect bees indirectly, via a variety of mechanisms. Two of the more well-studied mechanisms include reduced floral diet diversity and increased pesticide risk.

Simplified landscapes are often associated with reduced floral resource diversity and this effect can spill over to adjacent habitat types. For instance, floral species richness was reduced in Swedish grassland patches surrounded by increasing arable land (Öckinger, Lindborg, Sjödin, & Bommarco, 2012)

and insect-pollinated forb species richness along Irish dairy farm edges was reduced as surrounding grassland habitat area shrank (Power, Kelly, & Stout, 2012). Reductions in floral resource diversity have been shown to reduce bee species richness (Potts, Vulliamy, Dafni, Ne'eman, & Willmer, 2003), likely due to insufficient nutrition. Bees rely on a variety of floral species as sources of protein, carbohydrates, lipids, and amino acids (Donkersley et al., 2017; Roulston & Cane, 1999; Roulston, Cane, & Buchmann, 2000), and solitary bees rely on pollen mixtures of up to 6 plant families as a possible strategy to mitigate poor nutritional quality of a single floral resource. (Eckhardt, Haider, Dorn, & Muller, 2014). Thus, landscape simplification could negatively impact bees indirectly via reduced floral resource diversity in bee diets. Another mechanism through which landscape simplification could impact bee populations is pesticide risk (or pesticide exposure in terms of their toxicity to bees, in terms of LD_{50} values). Pesticide use can be higher in simplified landscapes, likely because of increased management in agriculture (Meehan, Werling, Landis, & Gratton, 2011, but see Larsen, 2013), which could lead to increased exposure to bees (but see Hladik, Vandever, & Smalling, 2016). There is abundant evidence that pesticide residues encountered by foraging bees through contact, inhalation, or ingestion from the air, water, soil, leaves, pollen, and nectar (Gradish et al., 2018) can have negative impacts. Pesticide risk at field-realistic levels can directly kill adult bees or reduce offspring production (Alston et al., 2007; Gill, Ramos-Rodriguez, & Raine, 2012; Rundlöf et al., 2015). But pesticides can also affect bee behavior, reducing foraging ability and homing success (Stanley et al., 2015), which could lead to more subtle, long-term impacts to offspring, such as reduced body size.

Most previous studies have investigated the impact of landscape simplification, diet diversity, and pesticide risk to bee populations in isolation, despite evidence that decreased diet diversity and increased pesticide risk can be consequences of landscape simplification. Moreover, there is evidence that diet diversity and pesticide risk are interlinked, where reduced floral diversity in honey bee-collected pollen corresponds with increased pesticide exposure in agroecosystems (Colwell, Williams,

Evans, & Shutler, 2017), potentially because less diverse diets contained more crop pollen. It is also possible that the combination of these two threats might lead to an exacerbated negative response in bees, as less nutritious diets might hinder bee ability to detoxify agrochemicals.

In order to maintain healthy pollinator populations in modern agroecosystems, it is essential we understand the mechanisms through which landscape simplification impacts bee populations, but few studies have addressed this question (but see Theodorou et al., 2016). Here, we aim to shed light on how landscape simplification impacts bees in agroecosystems, either directly, or via reduced floral diet diversity and increased pesticide risk. To address this question, we used a structural equations modeling framework to evaluate the direct, indirect, and interactive effects of 1) landscape simplification, 2) reduced floral diet diversity of bee pollen, and 3) increased pesticide risk in bee-collected pollen on solitary mason bee O*smia cornifrons* Radozkowski (Hymenoptera: Megachilidae) female offspring number and weight in New York apple orchards. We predict that all three threats will have negative effects on bee offspring number and weight via the following hypotheses (Fig. 2.1):

1) Landscape simplification will negatively affect offspring number and weight directly, through mechanisms not studied here, such as increased foraging trip time, parasitism, and/or predation.
2) Landscape simplification will negatively affect offspring number and weight indirectly via decreased diet diversity.
3) Landscape simplification will negatively affect offspring number and weight indirectly via increased pesticide risk levels in bee-collected pollen.
4) Reduced diet diversity will positively affect pesticide risk in pollen, because mono-floral crop pollen (apple pollen in this case) will more likely be contaminated with agrochemicals.

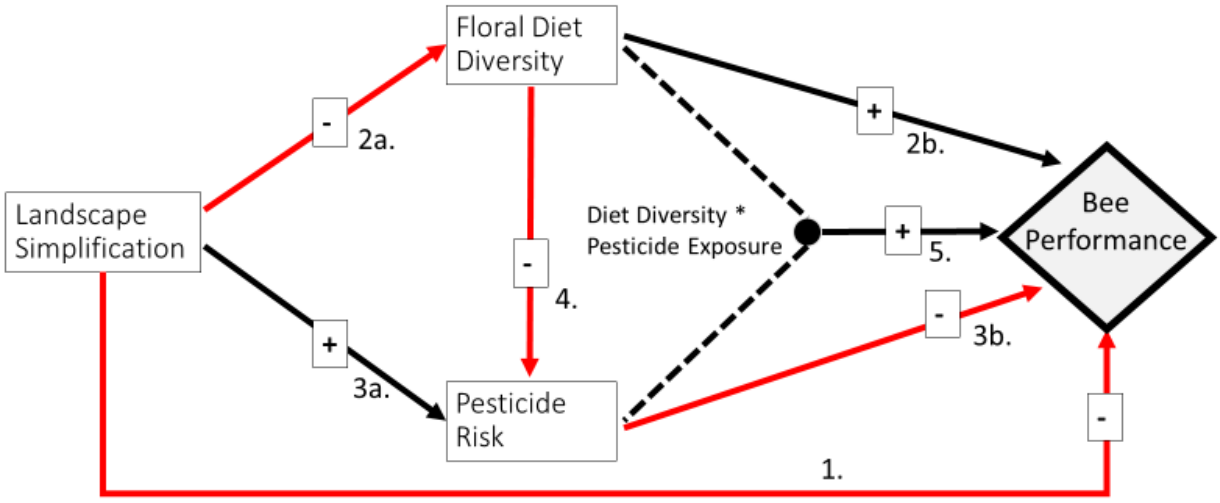


Figure 2.1. A schematic representation of our hypotheses (1-5, see Introduction) that landscape simplification will have a net negative correlation with bee offspring number and weight directly (1), or indirectly via diet diversity (2a and 2b) and pesticide risk (3a and 3b), and/or their combined effects (4, 5). Arrows represent unidirectional relationships between variables in boxes. Red arrows labeled with minus signs represent negative relationships, black arrows with plus signs are positive, and the dashed lines and circle represent a multiplicative interaction. Numbers and letters correspond to hypotheses in introduction, as well as the variable selection process described in the methods.

5) Reduced diet diversity and increased pesticide risk will synergistically interact to reduce offspring number and weight, because bees with homogenous diets will be less likely to overcome the toxic challenges of pesticide-laden pollen.

Materials and Methods:

Study System

Apple is an important crop for New York state and *O. cornifrons* is an apple pollinator (Maeta, 1990) that nests in trap-nests and responds to stress in measurable ways (Tepedino & Torchio, 1982). Although recently introduced from Japan, non-native *O. cornifrons* shares characteristics with many native bee species in apple: it is solitary, univoltine, polylectic, and mass-provisioning (Bosch & Kemp, 2001). Indeed, its potential for pollination services and management (Batra, 1978) make it an important species to study, especially because its population is increasing in areas where its native counterpart appears to be in decline (Bartomeus et al., 2013).

Study Design

We established 17 sites in the Finger Lakes region of NY (Fig. 2.2). Sites were at least 1065 m apart in privately-owned apple orchards ranging from 0.358 to 58.504 hectares (measured in qGIS3) and varying in proportion surrounding agriculture from 0.090 to 0.559 and proportion forest from 0.106 to 0.699 (Fig. 2.2, Fig. A2.4). Sites also varied in floral resource species richness in the environment from 62 to 361 flowering species per site in 2014 (Appendix A2.1), and in management from 0 to 78 sprays per orchard, encompassing 27 fungicides and 20 insecticides. We assessed pesticide risk, diet diversity, and bee performance at three time points that encompass the period were the bees were most active, with actual calendar dates varying per site (see Table A2.3 for dates). This resulted in 3 time points per 17 sites, totaling 51 time point observations per variable, unless otherwise stated.

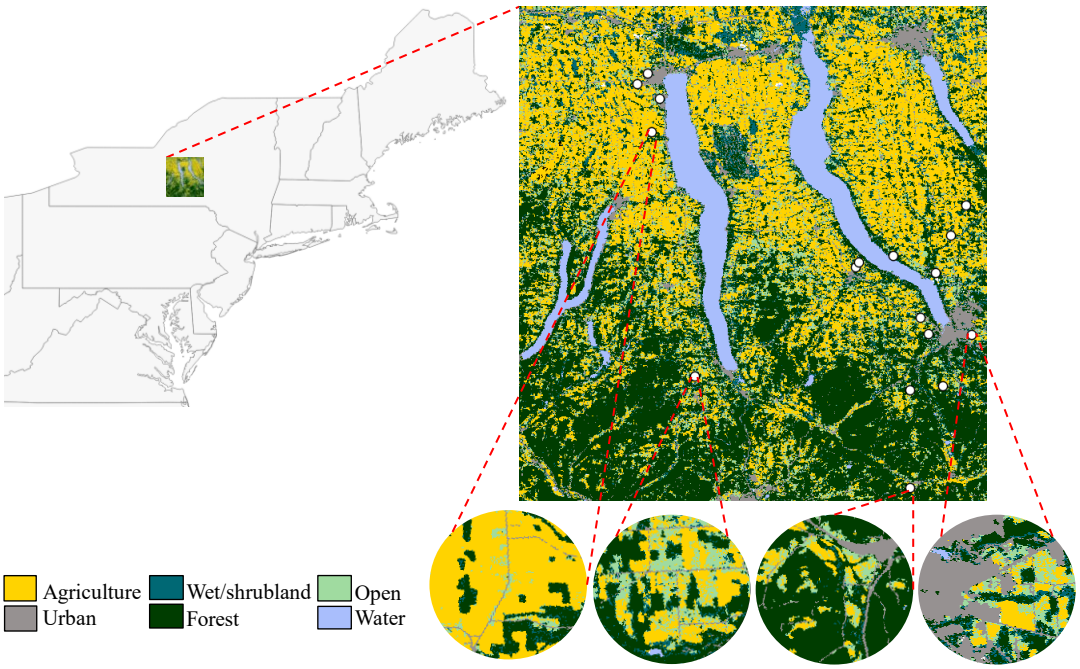


Figure 2.2. A map of the Finger Lakes Region of New York state (40.06 to 42.96 DD N, -76.13 to -77.14 DD W), showing the locations of the 17 orchard sites in our study (true geographic dimensions of the color insert are 83.516 km wide by 106.355 km tall). Map colors show the 5 landscape categories we created from the USDA 2015 Crop-Scape Data Layer, as well as open water. The variation in landscape composition amongst sites is shown here with 4 example sites at a 2 km radius (see Fig. A2.4 for landscape composition at all sites).

Landscape Composition

In order to find the most explanatory landscape variables for each hypothesized relationship (Fig. 2.1), we measured landscape composition at eight radii surrounding our 17 sites (250, 500, 750, 1000, 1250, 1500, 1750, and 2000 m). The smaller scales correspond to the 400 m or smaller radii at which there is 100 percent return rate of displaced *O. cornifrons* females to their nests (Kitamura & Maeta, 1969), but the maximum foraging range for *O. cornifrons* has not been determined and, across 4 species of *Osmia,* it varied between 150 and 1000 m (Gathmann & Tscharntke, 2002). In addition, the USDA Crop-Scape data layer which we used to measure landscape cover (see below) had a coarse (30 m raster) resolution, so its ability to predict land cover might only become accurate enough to correlate with *Osmia* response at scales larger than bees are flying. Also, bee visitation has been shown to vary with floral density, which in turn is dependent on patch size (Dauber et al., 2010), so the floral diet available to O. cornifrons might dependent on surrounding floral patchess, at scales that exceed their foraging radius. For some plant species, for instance, high density is only found when patch sizes exceed 2000 m^2 (Dauber et al., 2010). We used ArcGIS [ArcMap 10.5.1] and the USDA 2015 Crop-Scape Data Layer to quantify landscape simplification and landscape complexity. Landscape simplification was measured as the proportion surrounding agriculture and the proportion surrounding urban area, measured separately, because distinct agrochemicals are registered for private and commercial use and we would expect land management to differ between urban and agricultural habitats. Landscape complexity was measured as the proportion surrounding open area (wildflowers, grasslands, and pasture), the proportion forests, and the proportion shrub/wetlands surrounding the sites. These measures also could not be consolidated because we expect forests, shrub/wetlands, and open areas to vary in resource abundance, bloom time, and functionality to bee diets.

Experimental populations

Experimental populations were sourced from wild *O. cornifrons* by placing empty cardboard "trap-nest" nest tubes lined with paper (15.24 cm long by 7.5 mm diameter [crownbees.com]) at 6 privately-owned suburban backyards within the Town of Ithaca, NY (longitude: 42.428527 to 42.469463, latitude: -76.530422 to -76.465609 DD). We x-radiographed (*Agfa DX-G CR, Sound-Eklin Mark 1114 cw DR at* 52 kVp and 3.2 mAs) source nest tubes to determine the number of adult bees per nest and then randomly assigned 20-22 tubes containing a total of 98 to 102 adult *O. cornifrons* bees per experimental site. Our overall sex ratio averaged 59.4 ± 2.3% (mean ± standard error) female. Empty experimental nest tubes were housed in one wooden nest shelter per site, placed within the orchard or, if growers requested, on orchard perimeters, and were protected from predation with Tree Tanglefoot® and chicken wire (Fig. A2.2). To encourage nesting, source nest tubes were placed in nesting shelters coinciding with apple bloom (May 5, 2015) and were interspersed with 30 to 32 empty tubes (source tubes were marked). From bee emergence (May 7) until offspring production ceased (June 24), we collected newly completed nest tubes every six days and replaced them with empty tubes, always maintaining vacant nest tubes. To establish a precise nest construction time, only nest tubes completed between visits were collected. If the total nest tubes collected per time point were more than 1, additional tubes were set aside for pollen analysis (see *Pollen Analysis*). Temperature was collected hourly from inside mason bee nest shelters using data loggers [Embedded Data Systems iButtons].

Offspring Response

Nest tubes collected for offspring analysis (see Table A2.3 for numbers per time point) were stored, wrapped with wire mesh, and protected in a shed (42.444329, -76.462235 DD) at ambient temperature and then moved to a walk-in refrigerator on Cornell University campus for overwintering (at 3-4^{o}C) on November 18, 2015. Bees emerged in 51, 23 cm^3 collapsible mesh (mesh size < 1 mm^2) cages (at ~20 ^{o}C on a 9/15 hr light/dark cycle) per time point starting April 5 through April 20, 2016. Emerged bees were identified using microscopy and non-target species (1.04% *O. lignaria*) were

removed from our analyses. Because only 97 *O. cornifrons* specimens were collected in 22 NY apple orchards across 6 years (Russo, Park, Gibbs, & Danforth, 2015), or less than 1 specimen per orchard per year, the probability is low that local *O. cornifrons* established in nest tubes alongside seed bees.

Emerged offspring numbers were summed per cage (or time point) and the average wet weight [Mettler Toledo MS105DU Semi-Micro Balance] was taken for 10 males and 10 females (or however many existed if fewer) per time point (or cage). Due to two processing errors and three time points where no females emerged, we had only 46 time point observations for female weight. We chose the variables female offspring number and female offspring weight as our bee responses because: 1. Assessments on female offspring are more likely to reflect long-term population responses, as they produce offspring and provision nests, 2. When faced with environmental change, megachilid females have been shown to alter resource provisioning to female offspring without changing male provisions (Kim, 1999). Additional analyses for the number and weight of male offspring, as well as the proportion of females (or sex ratio), and larval mortality are included in Appendix A2.1, Fig. A2.1, and Table A2.1 in Appendix 2.

Pollen Analysis

If the total nest tubes produced at a time point were above 1 but below 10, then 1 nest tube was randomly selected for pollen analysis. When more than 10 nest tubes were produced, 2 to 4 of them were set aside for pollen analysis (see Table A2.3 for numbers). All other nest tubes were kept for offspring assessment. Nest tubes set aside for pollen analysis were frozen (-20 ^{o}C) immediately, to preserve pesticide residues and to kill bee eggs before pollen was consumed. To assess floral diet diversity and pesticide risk, pollen homogenates were created per time point by combining an equal amount of pollen (within 0.05 g) from each nest tube, ensuring equal representation per individual adult female nest.

Pesticide Risk

We screened pollen for the active ingredients of 188 pesticide active ingredients that were detectable using multi-residue liquid chromatography techniques. This list of chemicals was developed to capture as broad a range of active ingredients as possible in one analysis. It was also specifically developed to include many of the chemicals commonly used for management in specialty crops, including blueberries, pumpkin, cranberries, and apple, (Table 2.1, Table A2.2). Unfortunately, some of the more common agrochemicals (i.e. all pyrethroids and chlorothalonil), as well as two fungicides frequently used in apple (mancozeb and captan) could not be tested using multi-residue liquid chromatography (Stoner & Eitzer, 2013), and were not included in our analysis. Pesticides present in pollen were extracted using a modified version of the QuEChERS protocol (Stoner & Eitzer, 2013), analyzed with liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) and liquid chromatography/high resolution mass spectrometry (LC/HRMS). Detections were tested for quality using spiked samples (see Table 2.1 and Table A2.2 for quantification limits; see Stoner & Eitzer, 2013 for quality assurance tests conducted). To estimate the impact of pesticide risk (or the combination of exposure and toxicity) from the pollen provisions on bee offspring number and weight, we used the equation below, modified from Stoner and Eitzer (2013):

$$\%\, Hazard\ Quotient = \Sigma \frac{active\ ingredient\left(\frac{\mu g}{kg}\right) * \frac{pollen\ consumed\ (g)}{bee}}{LD50\left(\frac{\mu g}{bee}\right) * 1000} * 100$$

Pesticide risk was measured in percent hazard quotient (%HQ), which is the summation of the amount in parts per billion (ppb) of each agrochemical detected in the mean pollen consumed per bee, in terms of its toxicity (LD_{50}) per bee. To estimate mean pollen consumed per bee, we averaged the weight [Mettler Toledo AG245 Analytic Laboratory Scale] of 994 provision masses from the 109 of our experimental nest tubes set aside for pollen analysis (0.186 ± 0.004 mg). Because oral LD_{50} values and/or LD_{50} values

specific to *O. cornifrons* larvae were not available for all chemicals, we used acute (48 hr) topical LD_{50}s for honey bee adults. Thus, %HQ represents expected risk to *O. cornifrons*, but cannot predict actual mortality. We chose the lowest determined LD_{50} values from three sources (EPA, 2018; IUPAC, 2017; Tomlin, 2009). In cases where exact LD_{50} values were not available and the only information given was that the LD_{50} was "greater than" a tested value, we used the next highest whole number of the reported value. In both cases, this emulated the worst-case scenario, as we used the lowest possible LD_{50} value for hazard quotient estimates. Only fungicide and insecticide risk were estimated, as herbicides showed very low percent hazard quotients. Topical LD_{50} estimates were found for all pesticides detected above the LOQ (Table 2.1), except for 4-hydroxychlorothalonil, which was assumed to have the same topical LD_{50} value as its parent chemical (chlorothalonil). It should be noted that this likely over-estimates risk, as break-down products are expected to be less toxic than parent chemicals.

Diet Diversity

For each time point, 24 to 25 mg of the time point homogenate pollen was combined with 200 μL of water, vortexed for 15 s, and sonicated for 2 min to break up chunks of pollen. Ten μL of the resulting solution were pipetted onto microscope slides with 38 to 40 μL of Calberla's stain solution.

Using an Olympus BX41 compound light microscope at 40x magnification, we counted 300 pollen grains per slide within randomly-generated field-of-view transect(s), excluding obviously broken exines and grains that were not completely within the transect. Pollen grain morphotypes composing more than 3% (or more than 9 pollen grains) per sample were assessed for consistency by image, then identified to family level using pollen keys and image libraries (Girard, 2014; Kapp, Davis, & King, 2000; Russo, 2014), floral ranges and bloom times (USDA, 2017; Weldy, Werier, Nelson, Landry, & Campbell, 2017), and vouchered pollen slides collected from the environment surrounding our sites in 2014 (Appendix A2.1). We quantified floral richness, diversity (Shannon Index), and evenness at the family level. In addition, we

measured the proportion of the top four floral families collected in the pollen provisions (found at greater than 10 sites): Vitaceae, Rhamnaceae, Rosaceae (most likely apple, see Fig. A2.3), and Caprifoliaceae. Exhaustive floral resource surveys measuring species richness and abundance in the environment were conducted at 15 of the sites in 2014 (Appendix A2.1).

Statistical analysis

In order to evaluate our hypothesized relationships (Fig. 2.1) between landscape simplification, diet diversity, pesticide risk, and bee offspring and weight simultaneously, we adapted an information-theoretic approach (Burnham & Anderson, 2002) in two steps:

1) Variable selection:

In R, we used the dredge function (MuMln Package; Barton, 2018) to rank single predictor linear mixed effect models (lme function, nlme package; Pinheiro, Bates, DebRoy, & Sarkar, 2018) by the lowest corrected Akaike Information Criterion (AICc) value using maximum likelihood (Burnham & Anderson, 2002). All models included site as a random variable. For variable selection purposes, we assumed linear and normal model distributions. First, we selected the most explanatory predictor variable for all measures of landscape composition (Fig. 2.1:1), diet diversity (Fig. 2.1:2b), and pesticide risk (Fig. 2.1:3b), with female offspring number and weight as response variables. The list of potential variables for each hypothesized link (corresponding with Fig. 2.1) is presented below. We selected the most explanatory predictor variable from each of the following lists for steps 1, 2b, and 3b:

1) the most explanatory landscape scale (of 8) for proportion agriculture, forest, open, shrub/wetlands, and urban area

2b) floral Shannon diversity, floral richness, floral evenness, proportion Vitaceae, proportion Rhamnaceae, proportion Rosaceae, proportion Caprifoliaceae

3b) fungicide risk and insecticide risk

Next, we used the resulting, most explanatory diet diversity and pesticide risk variables from above (Fig. 2.1:2b and 3b, steps 2b and 3b) as responses of all measures of landscape composition. The list of potential variables for each hypothesized link (corresponding to Fig. 2.1:2a and Fig. 2.1:3a) is presented below. We selected the most explanatory predictor variable from this list for steps 2a and 3a.

2a/3a) the most explanatory landscape scale (of 8) for proportion agriculture, forest, open, shrub/wetlands, and urban area

Our selection process yielded the most explanatory predictor variables for each of our pair-wise, a-priori hypothesized relationships (Fig. 2.1: 1-5). See Table A2.4 for the top 3 predictor variables per relationship.

2) Confirmatory Path Analysis:

We used the selected variables from step one to construct two piece-wise structural equation (SEM) models (Shipley, 2009): one for female offspring number and one for female offspring weight. We chose piece-wise SEM because it can incorporate variables as both predictors and responses in the same analysis, allowing us to test for direct, indirect, and interactive effects simultaneously. It can also account for hierarchical data, or random effects. Site and time point were included as random and fixed variables, respectively, in all models. Path analysis models (lme function, nlme package) were constructed as follows:

a. bee offspring number and weight as the response with the following predictors: pesticide risk, diet diversity, the interaction term (diet diversity*pesticide risk) and landscape composition

b. diet diversity as the response with landscape composition as the predictor

c. pesticide risk as the response with landscape composition as the predictor

In order to minimize error, we included average temperature and time point as predictors of bee offspring number and weight, and the number of pollen provisions analyzed per time point (see Table A2.3 for temperature and pollen provisions per time point) as a predictors of diet diversity and pesticide risk. We accounted for covariance between female offspring weight and pollen provision number by including the relationships in our analysis as correlated error.

Path model residuals were graphically inspected to ensure that there were no violations of normality and homoscedasticity. Path model responses with non-normal distributions (skew less than -1.5 and greater than 1.5, kurtosis greater than 3.5) were transformed. The insecticide risk data had strong positive kurtosis, which was reduced with a log plus one transformation. Fungicide risk had both positive kurtosis and positive skew, which was rectified with a sixth-root transformation. Because the number of female offspring had strong kurtosis and were integer (count) data, we attempted Poisson and Quasi-Poisson models, but they were over-dispersed, so we instead used a square-root transformation which diminished the kurtosis. Female weight was negatively skewed with positive kurtosis, and thus squared, while proportion Rosaceae was right skewed and thus square-root transformed. Transformed models were tested for multicollinearity (VIF <4) and spatial autocorrelation ($-0.103 < r < 0.076$, $p > 0.100$) in the residuals using the Mantel test (ade4 package, Dray & Dufour, 2007).

The overall fit of path models was assessed by testing each independence claim using Shipley's d-separation test, and comparing observed correlations across independence claims to random variation using the Fisher's chi-square distributed C-statistic (Shipley, 2009). Statistics for path models were calculated and fitted by maximum likelihood methods using the piecewiseSEM package (Lefcheck, 2017). Initial path models were consistent with the data (number of female offspring: Fischer's $C=9.017$, $p=0.701$, $df=12$; female offspring weight: Fischer's $C=8.99$, $p=0.704$, $df=12$), but model fit, in terms of AIC-value, was improved (see Δ AIC in Fig. 2.3 caption) by iterative removal of non-significant ($p>0.1$) relationships

(Grace, 2006; Shipley, 2013). Results from path models are discussed here at the 90% ($p<0.1$) confidence level. Correlation coefficients were standardized by mean and standard deviation.

Generally, it is recommended for path analysis that the number of observations be at least 5 -fold the number of variables tested (Grace, Scheiner, & Schoolmaster, Jr., 2015). We therefore chose only one predictor variable for each hypothesized link, so we did not estimate more than 9 variables for each initial path (# obs. = 51, greater than 45). The number of observations for female weight (n=46) was only slightly above the cut-off for the number of parameters we estimated in our piecewise SEM. Thus, we performed a Monte-Carlo simulation, using the model parameters and our resulting chi-squared statistics from our initial path model to evaluate how likely it was that the observed value obtained could have arisen by random chance (CauseAndCorrelation package; Shipley, 2016, 2018). The fact that the Monte Carlo simulation chi-square probability was relatively high (0.7424) and that our final model for this response estimated even fewer parameters than our initial model (Monte Carlo X^2=0.924), is strong evidence that the results we present here were achieved with enough power.

In order to test the robustness of our results, we assessed the change in effect size estimates and significance when we replaced landscape variables in our final paths with adjacent scales. In light of our results, additional linear models were tested, using ANOVA, between the number of female offspring and female offspring weight, between apple orchard area and wet/shrubland, and between apple orchard area and proportion Rosaceae collected in provisions. Additional models included site and time point as random and fixed variables, respectively. Model normality and transformations were conducted as described above. All analyses were conducted using R 3.4.2 (R Core Team, 2017).

Results:

Across sites, the habitats with the largest landscape coverage at our largest, 2 km, radii were proportion forest, ranging from 0.106 to 0.699 (0.325 ± 0.042, n=17) and proportion agriculture, ranging from 0.090 to 0.559 (0.266 ± 0.037, n=17). Following these habitats in order of coverage were proportion urban (0.132 ± 0.033, n=17), open areas (mean 0.123 ± 0.009, n=17), and shrub/wetland (0.069 ± 0.006, n=17). See Fig. 2.2 and Fig. A2.4 for landscape composition. Of the 188 tested agrochemicals, 13 insecticides and 15 fungicides were found in the pollen provisions (see Table 2.1). Between 2 and 17 chemicals were detected per time point, ranging from 1.5 to 7,325.7 ppb (Table A2.5). Bees collected 11 floral families, between 2 and 7 (3.14 ± 0.16, n=51) per time point (Fig. A2.5). Of the 11 families, 4 were found at 10 or more sites and over 45% of time points: Vitaceae (where found: 0.451 ± 0.051, n=30), Rosaceae (where found: 0.365 ± 0.050, n=36), Caprifoliaceae (where found: 0.363 ± 0.043, n=44), and Rhamnaceae (where found: 0.273 ± 0.057, n=24). On average, 22 female offspring emerged per time point per site weighing 57.43 ± 0.09 mg (n=46; see Table A2.3). There was no evidence of a trade-off between female offspring number and weight; rather the two factors were positively correlated ($F_{1,28}$=9.044, p=0.0055, n=46; Fig. A2.7). Below, we discuss the resulting best-fit path models for female offspring number and weight.

Number of Female Offspring:

As shown in Fig. 2.3A and Table 2.2, increasing landscape simplification (proportion agriculture at 250 m) correlated with decreased floral Shannon diversity in bee-collected pollen (Fig. 2.4A), and reduced floral Shannon diversity corresponded with fewer female offspring (Fig. 2.4B), in accordance with our hypotheses (Fig. 2.1). As predicted, increasing agriculture at 2 km correlated with increased insecticide risk in pollen (Fig. 2.4C), but, contrary to our hypothesis, this did not correlate with any significant impacts on the number of female offspring. Instead, insecticide risk had a positive, albeit non-significant, relationship with the number of female offspring produced.

Table 2.1. Pesticide detection and risk calculation information. Of the 188 agrochemicals that were screnned using LC/MS/MS and LC/HRMS, 34 were detected above the average limit of quantification (LOQ), including 13 insecticides and 15 fungicides. Herbicide active ingredients, marked with italics below, were not included in risk estimation. Active ingredients are listed in order of the exposure (ppb) found across all 51 time points. Here, the average pesticide exposure (ppb) is shown for each active ingredient, along with the number and percent (parentheses) of the 51 time points where the ingredient was detected, as well as the average exposure (ppb) and minimum and maximum exposure where detected. Average LOQ values across three sets of samples are shown, as well as the acute (48 hr) adult honey bee topical LD_{50} values that were used in our analysis. LD_{50} values that were not exact in the literature are indicated in parentheses next to the selected LD_{50} value (see Methods). The LD_{50} value used for metabolite 4-hydroxychlorothalonil was the LD_{50} of its parent chemical, chlorothalonil. For each active ingredient, the average risk level (% hazard quotient, or %HQ), across time points is shown, as well as the percent contribution of each active ingredient to the total summed risk (%HQ) across all time points. Quantification limits are estimates based on pesticides spiked into 5 g honey bee pollen samples (due to limited *Osmia* samples) ranging from 20 to 30 ppb concentration and simultaneously analyzed with *Osmia* pollen. Due to differing size and nature of samples, these limits may vary from sample to sample. Pesticides not included in spiked samples are listed here as QLNT (Quantification Limit Not Tested). Most of the compounds with this designation have LOQs less than 5 ppb based on past 5 g bee-collected pollen samples. LOQ values for the 154 active ingredients that were tested and *not* detected can be found in Appendix 2 (Table A2.2).

active ingredient	type	ave exposure (ppb)	# and (%) of time points detected	ave exposure (min, max) where detected (ppb)	average LOQ (ppb)	topical LD_{50} (micrograms/bee)	ave risk (%HQ)	% of total risk (%HQ)
carbaryl	insecticide	268.65	26 (51%)	527.0 (27, 2289)	2.333	0.14	35.62	63.03
cyprodinil	fungicide	98.15	30 (59%)	166.9 (0.5, 1541)	10.000	785 (>784)	0.00	0.00
fluxapyroxad	fungicide	88.78	9 (18%)	503.1 (10, 1330)	QLNT	445 (>444)	0.00	0.01
pyraclostrobin	fungicide	85.52	12 (24%)	363.5 (6.1, 1000)	0.833	101 (>100)	0.02	0.03
indoxacarb	insecticide	43.92	5 (10%)	448.0 (134, 690)	1.000	0.068	11.99	21.22
difenconazole	fungicide	39.85	32 (63%)	63.5 (0.5, 876)	0.833	178 (>177)	0.00	0.01
trifloxystrobin	fungicide	34.89	19 (37%)	93.6 (0.5, 1120)	0.667	201 (>201)	0.00	0.01
boscalid	fungicide	30.22	3 (6%)	513.7 (179, 1131)	1.167	201 (>200)	0.00	0.00
iprodione	fungicide	27.31	12 (24%)	116.1 (2.7, 1099)	2.333	201 (>200)	0.00	0.00
methidathion	insecticide	13.69	2 (4%)	349.0 (298, 400)	QLNT	0.15	1.69	3.00
pyrimethanil	fungicide	11.54	5 (10%)	117.7 (7.4, 314)	6.000	101 (>100)	0.00	0.00
thiophanate-methyl	fungicide	10.33	5 (10%)	105.4 (14, 143)	QLNT	101 (>100)	0.00	0.00
metolachlor	*herbicide*	*8.32*	*50 (98%)*	*8.5 (0.5, 126)*	*0.500*	*111 (>110)*	*NA*	*NA*
chlorpyrifos	insecticide	7.33	7 (14%)	53.4 (6.6, 143)	2.000	0.059	2.30	4.08
atrazine	*herbicide*	*7.30*	*51 (100%)*	*7.3 (0.5, 73)*	*0.500*	*101 (>100)*	*NA*	*NA*
pendimethalin	*herbicide*	*6.41*	*21 (41%)*	*15.6 (0.7, 123)*	*0.667*	*100*	*NA*	*NA*
fenbuconazole	fungicide	5.76	10 (20%)	29.4 (3.4, 105)	2.000	293 (>292)	0.00	0.00
phosmet	insecticide	5.63	2 (4%)	143.5 (26, 261)	2.000	0.22	0.47	0.84
acetamiprid	insecticide	4.40	8 (16%)	28.0 (4.6, 72)	1.667	8.09	0.01	0.02
carbendazim	fungicide	3.20	4 (8%)	40.8 (15, 109)	1.333	51 (>50)	0.00	0.00
4-hydroxychlorothalonil	fungicide	3.16	9 (18%)	17.9 (2, 53)	2.000	182 (>181.29)	0.00	0.00
thiamethoxam	insecticide	1.25	11 (22%)	5.8 (1.4, 34)	1.000	0.024	0.96	1.71
thiacloprid	insecticide	1.16	10 (20%)	5.9 (1, 14.5)	1.000	17.94	0.00	0.00
diazinon	insecticide	1.08	34 (67%)	1.6 (0.1, 20)	0.567	0.13	0.15	0.27
myclobutanil	fungicide	0.56	2 (4%)	14.4 (3.7, 25)	1.667	33.9	0.00	0.00
penthiopyrad	fungicide	0.50	1 (2%)	26.0 (26, 26)	QLNT	501 (>500)	0.00	0.00
simazine	*herbicide*	0.35	4 (8%)	*4.4 (1.2, 14)*	*QLNT*	97	*NA*	*NA*
fenamiphos 1+2	insecticide	0.27	3 (6%)	4.6 (0.4, 13)	QLNT	0.28	0.02	0.03
spinosad	insecticide	0.22	1 (2%)	11.0 (11, 11)	5.000	0.0029	1.38	2.44
dichlorobenzamid	*herbicide*	*0.21*	*2 (4%)*	*5.4 (4.1, 6.6)*	*3.000*	*not found*	*NA*	*NA*
imidacloprid	insecticide	0.20	2 (4%)	5.0 (3.4, 6.6)	1.000	0.0038	0.96	1.69
clothianidin	insecticide	0.18	3 (6%)	3.0 (1.7, 4.8)	1.333	0.00368	0.90	1.59
fenuron	*herbicide*	*0.04*	*1 (2%)*	*1.4 (1.4, 1.4)*	*QLNT*	*not found*	*NA*	*NA*
dimethomorph 1+2	fungicide	0.02	1 (2%)	0.9 (0.9, 0.9)	0.500	102	0.00	0.00

Table 2.2. SEM analysis statistics for each bivariate relationship in the final structural equation models for the number of female offspring and female offspring weight. The response and predictor variables are listed along with their correlation coefficients, standard errors (se), degrees of freedom (df), sample size (n), critical values, p-values, significance levels, and transformations (response variables are listed first followed by predictor variables in order of appearance). Significance symbology is as follows: 0.05<p<0.1=no symbol, 0.01<p<0.05=*, 0.001<p<0.01=**, p<0.001=***. Variables in italics represent correlated errors.

SEM Model	Response	Predictor	se	Df	n	Critical Value	p-value	Correlation Coefficient	Significance	Transformation(s)
Number Female Offspring	Insecticide Risk (%HQ)	Agriculture 2000 m	1.999	15	51	3.203	0.006	0.480	**	log+1
	Insecticide Risk (%HQ)	Time Point	0.224	31	51	2.904	0.007	0.266	**	log+1
	Insecticide Risk (%HQ)	Temperature	0.059	31	51	1.867	0.071	0.206		log+1
	Insecticide Risk (%HQ)	Number Pollen Provisions	0.032	31	51	-2.534	0.017	-0.274	*	log+1
	Floral Shannon Diversity	Agriculture 250 m	0.166	15	51	-2.680	0.017	-0.417	*	
	Floral Shannon Diversity	Temperature	0.010	32	51	0.982	0.333	0.128		
	Floral Shannon Diversity	Number Pollen Provisions	0.005	32	51	1.196	0.240	0.156		
	Number Female Offspring	Floral Shannon Diversity	1.170	29	51	3.049	0.005	0.486	**	square-root
	Number Female Offspring	Insecticide Risk (%HQ)	0.313	29	51	0.025	0.980	0.008		square-root, log+1
	Number Female Offspring	Time Point	0.247	29	51	-3.559	0.001	-0.349	**	square-root
	Number Female Offspring	Temperature	0.053	29	51	3.128	0.004	0.300	**	square-root
	Number Female Offspring	Floral Shannon Diversity: Insecticide Risk (%HQ)	0.405	29	51	-1.359	0.185	-0.393		square-root, log+1
	Number Female Offspring	*Number Pollen Provisions*	*NA*	*51*	*51*	*2.602*	*0.006*	*0.352*	***	*square-root*

Table 2.2 (Continued).

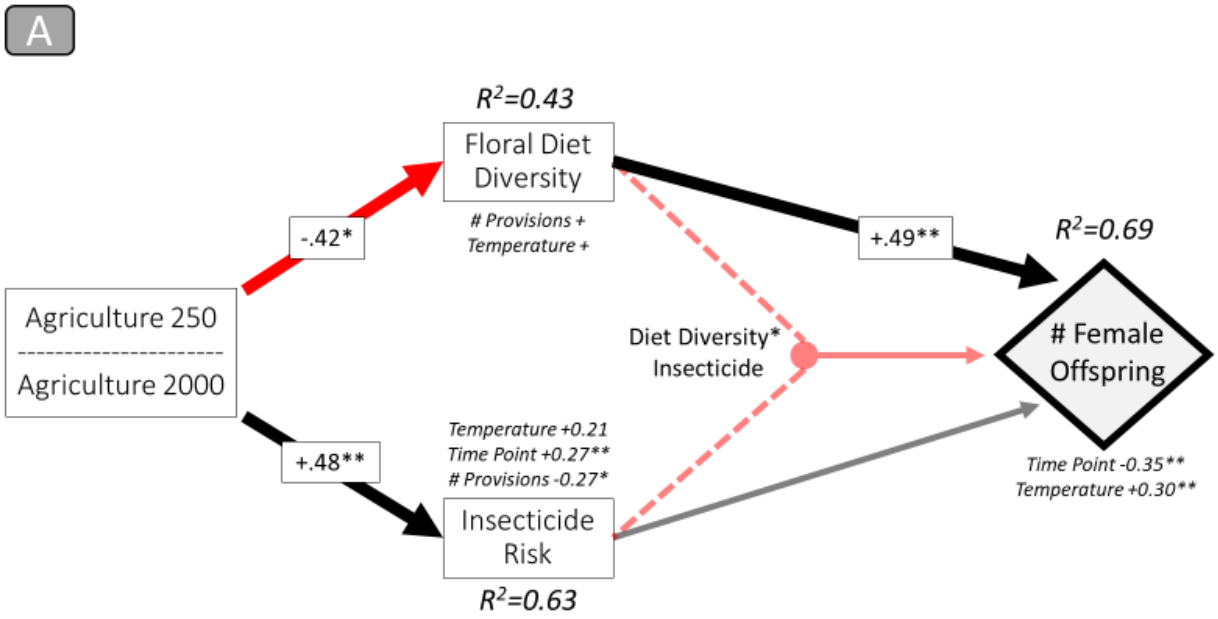
SEM Model	Response	Predictor	se	d.f.	n	Critical Value	p-value	Correlation Coefficient	Significance	Transformation(s)
Female Offspring Weight	Female Offspring Weight	Fungicide Risk (%HQ)	0.000	27	46	-2.032	0.052	-0.261		squared, 6th-root
	Female Offspring Weight	Open 2000 m	0.003	15	46	2.926	0.010	0.376	*	squared
	Female Offspring Weight	Temperature	0.000	27	46	2.866	0.008	0.342	**	squared
	Fungicide Risk (%HQ)	Proportion Rosaceae	0.063	26	46	1.895	0.069	0.146		6th-root, square-root
	Fungicide Risk (%HQ)	Agriculture 500 m	0.226	15	46	2.921	0.011	0.585	*	6th-root
	Fungicide Risk (%HQ)	Temperature	0.003	26	46	1.638	0.114	0.075		6th-root
	Fungicide Risk (%HQ)	Time Point	0.014	26	46	-1.903	0.068	-0.084		6th-root
	Proportion Rosaceae	Shrub/wetland 1250 m	2.058	15	46	-2.904	0.011	-0.516	*	square-root
	Proportion Rosaceae	Time Point	0.038	28	46	-1.857	0.074	-0.178		square-root
	Female Offspring Weight	*Number Pollen Provisions*	*NA*	*46*	*46*	*0.675*	*0.252*	*0.102*		*squared*

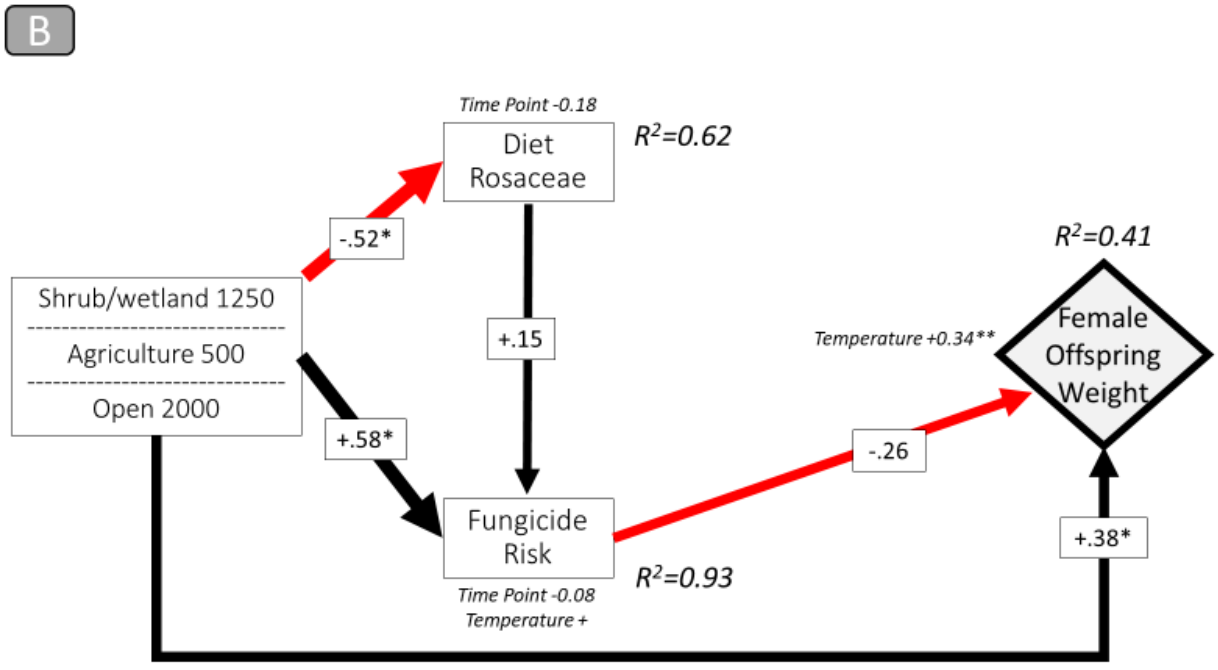
Also contrary to our hypothesis (Fig. 2.1), high floral Shannon diversity in the pollen did not overcome the negative effects of high insecticide risk (Fig. 2.3A). Counter to our hypothesis, there was no synergistic interaction between insecticide risk and floral Shannon diversity, and no correlation between insecticide risk and the number of female offspring produced. In contrast, increasing floral Shannon diversity positively correlated with increased female offspring (Fig. 2.3A), and was a highly significant and explanatory predictor ($p=0.005$, $R^2=0.48$).

Female Offspring Weight:

As shown in Fig. 3B and Table 2.2, female offspring weight directly increased with increasing landscape complexity, in the form of open areas at 2000 m around sites (Fig. 2.5A). Increasing landscape complexity also had an indirect positive correlation with female weight. Increasing proportion shrub/wetlands at 1250 m indirectly corresponded with heavier females via its correlation with reduced proportion Rosaceae in pollen (Fig. 2.5B) and its negative, marginally significant correlation with fungicide risk (Fig. 2.5C). Fungicide risk, in turn, was marginally correlated with reduced female offspring weight (Fig. 2.5D). Conversely, increasing landscape simplification, or proportion agriculture at 500 m, indirectly resulted in lighter females via its correlation with increased fungicide risk in pollen (Fig. 2.5A and 2.5D). Orchard area, in hectares, did not correlate with an increase in the proportion shrub/wetland at 1250 m ($F_{1,15}=0.342$, $p=0.567$, $n=17$), indicating that the decrease in Rosaceae pollen collected was not a product of reduced orchard size. As expected, our results show a positive correlation between floral diet and pesticide risk. Increasing proportion Rosaceae marginally correlated with high fungicide risk in bee-collected pollen, probably resulting from sprays in apple. Orchard area correlated positively with increasing proportion Rosaceae in provisions, suggesting it is highly likely that our category “Rosaceae” includes mostly apple pollen ($F_{1,15}=7.88$, $p=0.013$, $n=51$; Fig. A2.3). Interestingly, on its own, high proportion Rosaceae did not have an effect on female weight, but was associated with reduced offspring weight only through its marginal correlation with higher fungicide risk.

Figure 2.3. Resulting final path models for the number of female offspring (A; Fisher's C=7.956, p=0.789, df=12, n=51, Δ AIC from initial model=-7.061) and female offspring weight (B; Fisher's C=14.813, p=0.675, df=18, n=46, Δ AIC from initial model=-6.177). Unidirectional arrows represent supported relationships (red negative, black positive) between variables (in boxes). Links found in a priori model may be omitted here because their removal increased model fit. Arrows are scaled to the magnitude of the standardized correlation coefficients, shown in boxes alongside arrows accompanying p-value significance levels (0.05<p<0.1=no symbol, 0.01<p<0.05=*, 0.001<p<0.01=**, p<0.001=***). Semi-transparent arrows represent non-significant (p>0.1) relationships that still supported the model fit (based on AIC score). For clarity, the variables "Number of Pollen Provisions" (# Provisions), "Temperature", and "Time Point" have been omitted and instead their correlation coefficients are shown in italics next to boxes of associated response variables (magnitude not shown for non-significant relationships). Numbers next to landscape categories represent the scale, or radius in meters about sites. Statistics are based on transformed variables (see Table 2.2).


A
R^2=0.43
Floral Diet Diversity
Provisions +
Temperature +
-.42*
+.49**
R^2=0.69
Agriculture 250
Agriculture 2000
Diet Diversity*
Insecticide
Female Offspring
+.48**
Temperature +0.21
Time Point +0.27**
Provisions -0.27*
Insecticide Risk
R^2=0.63
Time Point -0.35**
Temperature +0.30**


B
Time Point -0.18
Diet Rosaceae
R^2=0.62
-.52*
R^2=0.41
Shrub/wetland 1250
Agriculture 500
Open 2000
+.15
Temperature +0.34**
Female Offspring Weight
+.58*
-.26
Fungicide Risk
+.38*
R^2=0.93
Time Point -0.08
Temperature +

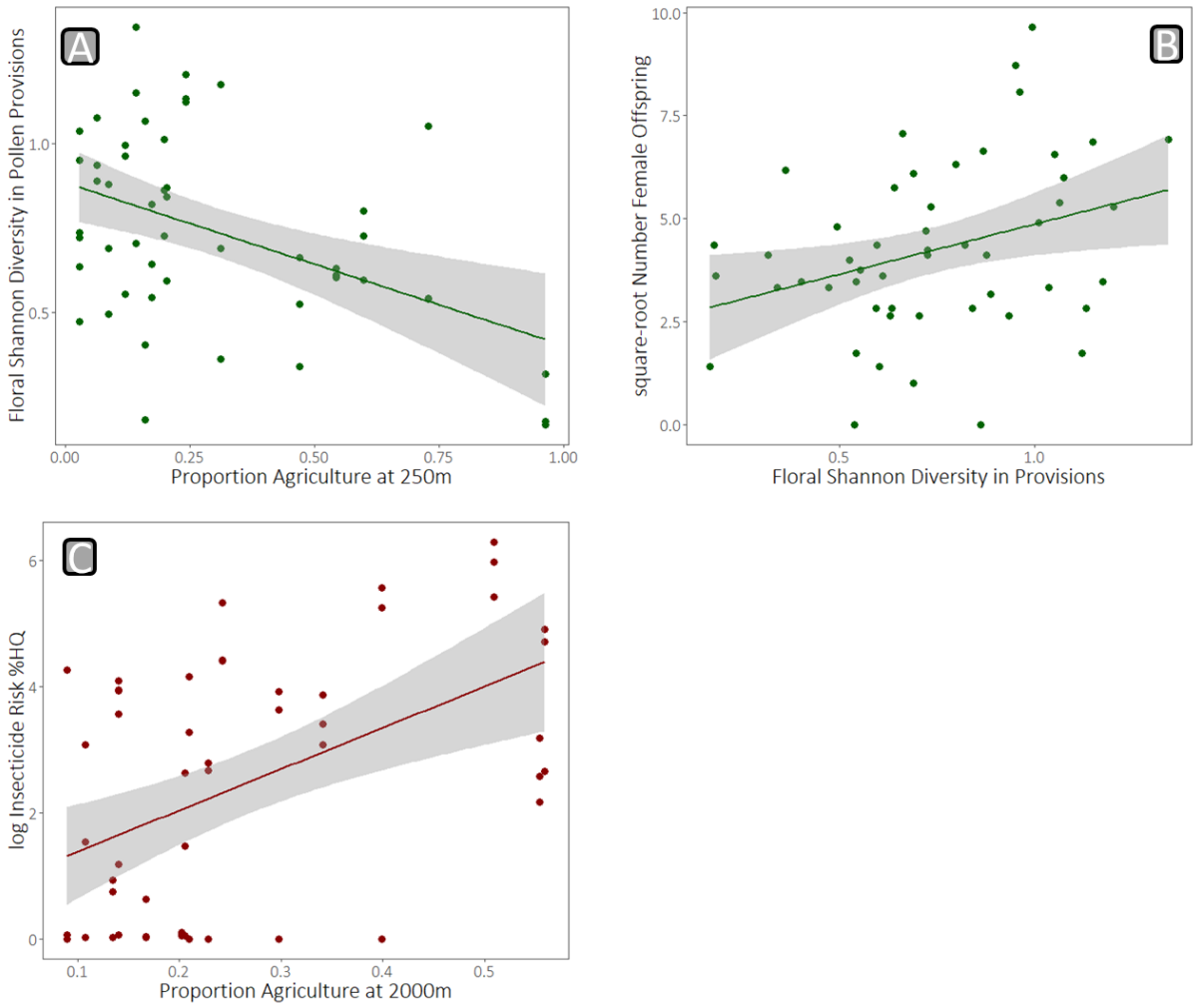


Figure 2.4. The three significant pairwise relationships found in the path analysis for the number of female offspring. Here, we show the relationships between agriculture at a 250 m and floral Shannon diversity at the family level in bee-collected pollen provisions (A; p=0.017, n=51), between floral Shannon diversity and the square-root of the number of female offspring produced (B; p=0.005, n=51), between agriculture at 2000 m and the logarithm of insecticide risk (% hazard quotient) in pollen (C, p=0.006, n=51), Points show time-point observations, lines denote relationships between variables, and grey shadows represent 95% confidence intervals. Plots show single predictor data with no random variables (See Table 2.2 for multi-modal SEM statistics).

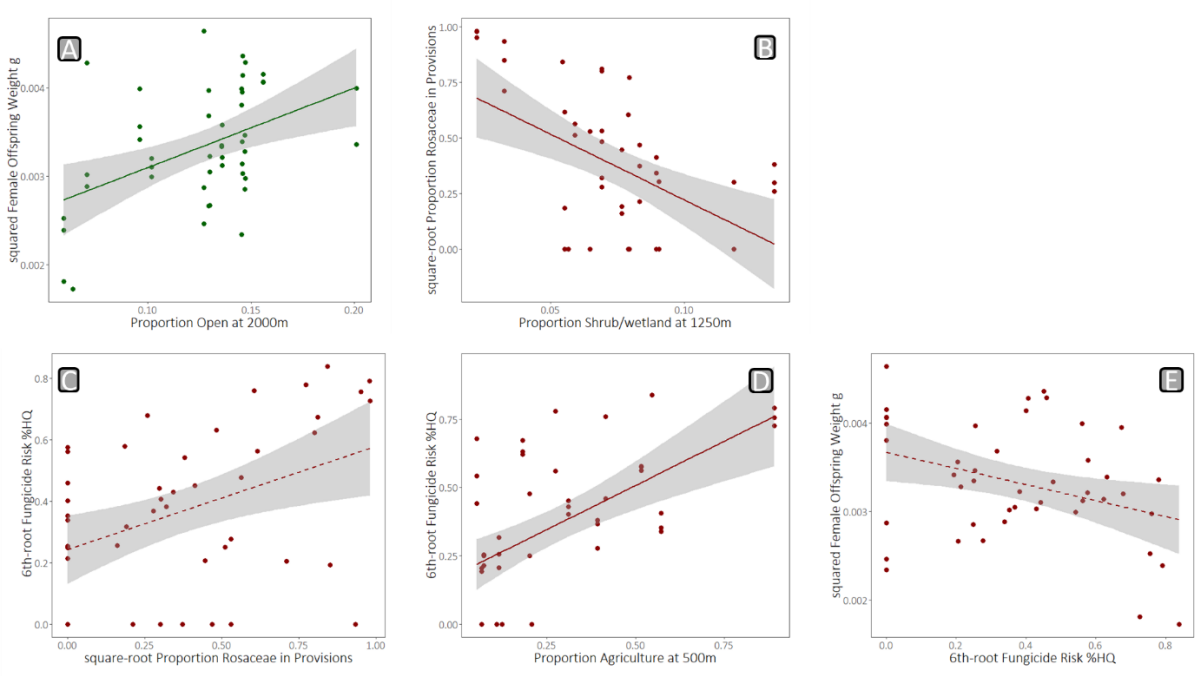


Figure 2.5. The pairwise relationships found in the path analysis for female offspring weight, including the relationship between open area at 2000 m and squared female offspring weight (A; p=0.010, n=46), the relationship between proportion shrub/wetlands at 1250 m and the square-root of the proportion Rosaceae in bee-collected pollen provisions (B; 0.011, n=46), the marginal relationship between the square-root of the proportion Rosaceae and the 6th-root of the fungicide risk in bee-collected pollen provisions (C; p=0.069, n=46), the relationship between the proportion agriculture at 500 m and the 6th-root of fungicide risk in pollen, measure as percent hazard quotient (D; p=0.011, n=46), and the marginal relationship between the 6th-root of fungicide risk and squared female offspring weight in grams (E; p=0.052, n=46). Points show time-point observations, lines denote relationships between variables, and grey shadows represent 95% confidence intervals. Plots show single predictor data with no random variables (See Table 2.2 for multi-modal SEM statistics).

Robustness of Results:

Changes in scale of landscape variables resulted in minimal changes to effect size estimates (ranging from 0.002 to 0.013 per path), and effect size direction remained stable. Changes in p-values averaged 0.013 to 0.07 per path, resulting in marginal changes in significance of relationships (see Table A2.6).

Discussion:

In accordance with our hypotheses (discussed below), we found that landscape simplification, in the form of increased proportion agricultural area surrounding our sites, resulted in fewer and smaller female *Osmia cornifrons* offspring, via its correlation with increased fungicide risk, reduced floral diversity, and the marginal correlation between them. Contrary to our expectation, we found no evidence that diverse pollen diets buffer bees from the impacts of pesticide risk, nor did we find evidence that insecticide risk had negative effects on bee response.

Hypothesis 1: Landscape simplification reduces offspring number and weight directly

As open areas (landscape complexity) increased at a 2 km radius, female offspring weighed more, consistent with previous work showing that grasslands and meadows support higher bee abundance (Hines & Hendrix, 2005; Moroń et al., 2008). This effect on bee offspring weight was not explained by floral diet or pesticide risk, suggesting a direct effect of landscape simplification on bee weight. One possible explanation is that open habitats support reduced pathogen infection. In a separate study, our offspring were screened for the common fungal pathogen: chalkbrood, or *Ascosphaera*. Increasing open areas, surrounding our sites correlated with reduced larval mortality due to *Ascosphaera* (Krichilsky et al., in prep). It could be that offspring from our study were infected with *Ascosphaera* or some other pathogen as larvae, but this infection did not result in direct mortality, and

instead simply reduced their weight upon emergence. Another possibility is that open areas could support higher floral resource abundance, which could reduce adult female foraging trip time (Jha & Kremen, 2013; Westphal et al., 2006). This may have corresponded to larger pollen provisions, and thus, heavier females. It has also been shown that increased bloom density around *Osmia pumila* nests correlates with reduced nest predation and parasitism by the brood parasitic wasp, *Sapyga centrata* (Goodell, 2003), which might indicate that our female offspring were heavier because their mothers faced reduced predation and parasite pressure.

Hypothesis 2: Landscape simplification reduces offspring number and weight indirectly via reduced diet diversity

We found that increasing proportion agriculture at 250 m, well within the foraging range of female *O. cornifrons* (Kitamura & Maeta, 1969), corresponded with reduced floral Shannon diversity in bee-collected pollen, and this correlated with fewer female offspring produced. We did not find a relationship between floral Shannon diversity and floral resource abundance in the environment in the 2014 surveys (Fig. A2.6), which suggests that reduced floral Shannon diversity alone, and not abundance, limited the number of offspring produced. Indeed, polylectic *Osmia* have been shown to rely on a mixture of floral resources to maintain stable protein content in pollen provisions (Lunau & Budde, 2007).

Although we could not identify individual Rosaceous pollens, increasing orchard area correlated with a higher proportion of Rosaceae pollen in provisions (Fig. A2.3), suggesting that a majority of Rosaceae pollen was apple, or crop, pollen. We found that landscape simplification in the form of decreasing shrublands and wetlands at 1250 m corresponded with smaller female offspring, via an increase in Rosaceae-heavy pollen provisions (and their marginal correlation with fungicide risk). Perhaps this increase in Rosaceae collected could be because orchards, and the resources they provide, are larger in drier landscapes, when shrub/wetland cover is low, but this was not the case (see Results),

suggesting instead that some property of declining shrub/wetlands correlates with increased Rosaceae collection. Seeing smaller female offspring in response to higher proportion Rosaceae in the pollen could be because mono-floral, majority apple, pollen does not represent an ideal diet. Indeed, *Osmia* rely on a diversity of shrubland resources (Kraemer & Favi, 2005) and shrubland habitats were the second-best predictor of high floral Shannon diversity in our study (Table A2.4). Six of the 11 pollen types collected were determined to the genus or species level and identified as the shrubland plants buckthorn, honeysuckle, dogwood, privet, viburnum, and walnut. Though shrublands seem to dictate bee pollen collection at a scale much larger than their established foraging range, this could be explained by flowering shrub species that rely on a critical area of surrounding shrubland habitat to maintain populations (Dauber et al., 2010). Past work corroborates our findings, showing that *Osmia* collect relatively more Rosaceae pollen and produce smaller provisions as surrounding natural habitats become more limited (Nagamitsu et al., 2017).

Hypothesis 3: Landscape simplification reduces offspring number and weight indirectly via increased pesticide risk

As proportion agriculture surrounding bee nest shelters increased, female offspring weighed less, via their marginal relationship with increasing fungicide risk in pollen. Fungicide risk responded to agriculture at a 500 m radius, consistent with the less than 600 m radius orchard size at our sites, making it likely that fungicides in pollen came from apple management. Past research in apple shows that increased fungicide use reduces bee species richness and abundance, and that this effect is exacerbated in simpler, agriculture-dominated, landscapes (Park, Blitzer, Gibbs, Losey, & Danforth, 2015). Here, we show that, in the case of *O. cornifrons*, this interaction could be driven by landscape simplification, or increasing agriculture, correlating with increased fungicide risk, indirectly, marginally, reducing offspring weight and likely leading to population reduction over time (see Implications).

We also found that increasing landscape simplification, in the form of agricultural area, corresponded with increased insecticide risk in the pollen, but this did not translate to fewer female offspring. In contrast to fungicides, insecticide risk positively correlated with agriculture at 2 km. This large scale suggests that insecticides might be impacting bees outside of the focal orchards. In fact, past research shows that the majority of pesticide exposure in honey bee-collected pollen from New York apple orchards came from insecticides sprayed outside the orchards or prior to bloom (McArt, Fersch, Milano, Truitt, & Böröczky, 2017).

Though the insecticide hazard quotients exceeded 100% of the LD_{50} at 8 of 51 time points, we did not see fewer female offspring in these cases. However, higher fungicide risk resulted in lighter female offspring. One explanation for why we do not see an effect of insecticide risk on the number of female offspring could be because female eggs were laid before insecticide risk was high. Growers tend to spray more fungicides during apple bloom and switch to insecticides after bloom (Park et al., 2015), and mason bees usually provision female offspring before males. Our results support this idea, as insecticide risk in pollen increased as time point progressed (Fig. 2.3A), and we found a significant, negative correlation between insecticide risk and the number of male offspring, which were produced later in the season. (Fig. A2.1). It is also possible that insecticides had a more significant impact female offspring number than were able to detect here, because we did not screen for commonly used insecticides such as pyrethroids or chlorothalonil (see Methods).

Hypothesis 4: Reduced diet diversity will positively affect pesticide risk in pollen

For our results on female weight, we found support for the predicted pattern. Fungicide risk in pollen marginally increased as diets became more mono-floral, in this case as proportion Rosaceae in pollen increased. This was likely due to pesticide sprays in apple orchards, because the proportion of Rosaceae pollen increased as apple orchard area expanded (Fig. A2.3). Even Rosaceae pollen that was not apple was likely subject to apple agrochemical sprays, as we noted many Rosaceous plants in

orchards included other cultivated fruits (*Prunus* and *Fragaria*) or creeping plants (*Potentilla, Geum, Rubus,* and *Rosa)* found in or around the orchards. It is possible that the relationship between female offspring weight and fungicide risk might be even more pronounced than what was found here, as we were not able to screen for captan and mancozeb, two of the more common fungicide active ingredients used for apple management.

However, we did not find the same pattern for insecticide risk in the path analysis on the number of female offspring. In this case, there was no relationship between diverse diets, measured as floral Shannon diversity at the family level, and decreased insecticide risk. Perhaps insecticide contamination in this case was coming from other sprays outside of apple. Indeed, apple growers tend to spray more fungicides than insecticides in early spring (Park et al., 2015), and past study of honey bees in our orchards showed that fungicide exposure increased with increasing apple pollen, while insecticide exposure did not (McArt et al., 2017). In fact, in the same study, insecticide risk in honey bee pollen *increased* with increasing floral richness in their pollen (McArt et al., 2017), making our result with mason bees less surprising.

Hypothesis 5: Reduced diet diversity and increased pesticide risk will synergistically interact to reduce female offspring number and weight

Contrary to our hypothesis, we saw no evidence of a synergistic effect of diet diversity and pesticide risk on bee response. The lack of a synergistic interaction between Rosaceae-heavy diets and fungicide risk indicates that the indirect, negative effect of proportion Rosaceae pollen on bee weight is mostly due to fungicide risk, and not indicative of decreased nutrition associated with a more mono-floral diet, as we expected. Similarly, contrary to our expectation, there was no evidence of a "rescue effect" (or synergism) between diet diversity and insecticide risk on the number of female offspring produced. However, there was *also* no evidence of a negative effect of insecticide risk on the number of females (see above discussion), suggesting that we cannot rule out the possibility of a rescue effect

under different circumstances. But the potential for a rescue effect in females does seem unlikely, as we also saw no rescue effect of diet diversity and on the number of males produced, despite that fact that insecticide risk had a significantly negative correlation with the number of male offspring (Fig. A2.1A).

Implications:

Here, we show that landscape simplification (via increased proportion agriculture and reduced open areas and shrub/wetlands) in agroecosystems corresponds with reductions in solitary bee female offspring number and body size, through its correlation with reduced diet diversity, its marginal correlation with increased pesticide risk, and the marginal correlation between them in pollen provisions. We also show that diet diversity did not appear to mitigate the negative effects of insecticide risk, and that insecticide risk did *not* have a negative effect on the number of offspring.

Based on our results, we would expect that increasing floral resource diversity in the environment (through wildflower plantings or reduced mowing), conserving complex landscapes, and reducing fungicide risk (through IPM and development and use of new pest control techniques) would support *O. cornifrons* populations in apple. However, further research with additional species in multiple cropping systems is imperative to inform management strategies for wild bees in agroecosystems. Even though *O. cornifrons* shares characteristics with many wild, solitary bees in apple, it is non-native and enjoys a stable population, while many of its native counterparts have narrower diet breadths, have smaller body size, and are currently in decline (Bartomeus et al., 2013). Thus, we suspect that the population response of this introduced, non-native species may be a conservative estimate of how native bees might respond to similar stress. Additionally, our results here only focus on the implications for female offspring, though we know that male offspring also showed reduced weight and number in response to landscape simplification, pesticide risk, and diet diversity (Fig. A2.1). The potential impacts on mason bee populations may in fact be even larger when male responses are considered.

Because we could not count adult females in the field, finding fewer *O. cornifrons* offspring could result from: 1) reduced per capita offspring production, 2) direct adult female mortality, or 3) adult female dispersal to more favorable locations (Bosch & Kemp, 2001). Regardless of the mechanism, *O. cornifrons* populations, and thus their pollination services, were reduced in simplified landscapes. Not only did we find fewer female offspring in simpler landscapes, but they also weighed marginally less. Reduced intra-specific female body size in bees can lead to decreased offspring production, slower provisioning rates, reduced longevity (Bosch & Vicens, 2006; Kim, 1997), and even lead to less effective pollination (Jauker et al., 2016). We must continue to research the simultaneous impact of multiple variables to understand how landscape simplification impacts pollinator health across a range of species and cropping systems to ensure that our important pollinator species are not unwittingly handicapped in the very environments where we rely on them.

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CHAPTER 3

CROPLAND COVER DRIVES PESTICIDE RISK TO MASON BEES BUT NOT HONEY BEES IN APPLE ORCHARDS

Abstract:

Both honey bees and wild bees are important pollinators in agroecosystems, but pesticide risk from crop pollen can negatively impact bee performance and pollination services. Honey bees are often used as a model system to assess pesticide risk to all bees, despite the fact that they have distinct life-histories compared to many wild bees. Here, we compare pesticide exposure and risk in wild mason bee (*Osmia cornifrons*) pollen provisions and honey bee (*Apis mellifera*) bee bread from nests and colonies placed in the same 14 apple orchards during bloom. We investigate how apple land cover, *Malus* pollen collection, and the most predictive apple land cover radius at which bees collected *Malus* pollenmight influence pesticide exposure and risk. As predicted, we found that mason bees collected a greater mean proportion *Malus* pollen than did honey bees. While mason bee pollen provisions contained greater mean pesticide residues (ng/g) and higher pesticide risk (% Hazard Quotient) levels than honey bee beebread, the differences between species were not significant. We also found that apple land cover was associated with mason bee pesticide risk, via increasing proportion *Malus* pollen collected, while apple land cover did not influence exposure or risk to honey bees. Our results suggest that, while there were no significant differences in pesticide exposure and risk in bee-collected pollen between species, exposure and risk for mason bees was largely driven by cropland cover and crop pollen collection, likely due to their smaller *Malus* foraging radius and greater *Malus* pollen collection. Because many wild bees are diet specialists and have an even smaller foraging range than O. cornifrons, we are in danger of

underestimating the potential influence of cropland and crop pollen on wild bees when we use *A. mellifera* as a model of pesticide risk for wild bee pollinators.

Introduction:

Recent declines in both wild and managed bees have sparked concerns about bee health and the services that pollinators provide. One of the main hypothesized contributors to bee decline in agroecosystems is exposure to harmful pesticides (Goulson, Nicholls, Botías, & Rotheray, 2015; Potts et al., 2010; Vanbergen, 2013). Pesticides can negatively influence bee health and the pollination services provided by bees. For example, pesticide exposure in agroecosystems can increase honey bee worker mortality and queen failure in corn fields (Tsvetkov et al., 2017), can reduce bumble bee colony biomass and queen weight (Bernauer, Gaines-Day, & Steffan, 2015), and can reduce wild bee nesting and progeny production in apple orchards (Alston et al., 2007). In addition, pesticide exposure can also have more subtle effects, such as reducing pollen collection (Gill, Ramos-Rodriguez, & Raine, 2012) or nest recognition by wild bees (Artz & Pitts-Singer, 2015). Direct bee mortality and population reductions could significantly affect pollination services, and even the more subtle responses to pesticide exposure, like reduced intraspecific body size and reduced foraging efficiency, have been correlated with decreased crop yield (Jauker, Speckmann, & Wolters, 2016; Stanley et al., 2015).

Although bees can be exposed to pesticides through many routes (Gradish et al., 2018), it is largely thought that most pesticide exposure to bees occurs through their interactions with crop pollen. For example, honey bees foraging in apple, cranberry, and maize fields were exposed to higher pesticide concentrations in their pollen compared with bees foraging in non-agricultural areas (Colwell, Williams, Evans, & Shutler, 2017; Long & Krupke, 2016), presumably because bees were collecting crop pollen, which typically has greater pesticide concentrations than adjacent wildflower pollen (David et al., 2016).

If crop pollen is the main delivery route of pesticide exposure to bees, then crops with larger land cover should pose an even greater threat. Studies have shown that, in sites with higher proportion surrounding cropland, bees collected higher proportions of crop pollen and, thus, higher concentrations of pesticides in their pollen (Balfour et al., 2017; McArt, Fersch, Milano, Truitt, & Böröczky, 2017). These results suggest that bees are most threatened by pesticides in the very areas where we rely most heavily upon their pollination services.

Despite the importance of wild bees to pollination services and the fact that pesticides are known to negatively impact wild bee health in agroecosystems, most of the studies assessing pesticide exposure to bees in crops are conducted with honey bees. There are numerous surveys quantifying pesticide concentrations in honey bee wax, pollen, and honey near crops (Chauzat et al., 2006; Frazier et al., 2015; Long & Krupke, 2016; Lu, Chang, Tao, & Chen, 2015; Mitchell, Mulhauser, Mulot, & Aebi, 2017; Mullin et al., 2010). In contrast, surveys of wild bee nesting materials are scarce (but see Woodcock et al., 2017; Centrella et al., in prep). In fact, because they are easier to study and manage than most wild bees, *Apis mellifera* are often used as model organisms and have historically been the sole species used to estimate the impact of pesticides to all bees (EPPO/OEPPO, 2009; Sgolastra et al., 2018a; US EPA, 2019).

Nonetheless, *Apis mellifera* is likely a poor model for estimating pesticide risk to wild bees. Pesticide risk accounts for different toxicities of different agrochemicals and is measured as exposure to an agrochemical (in ppb) *in terms* of the toxicity (using LD/LC_{50} values) of that agrochemical to bees (Stoner & Eitzer, 2013) . Indeed, a meta-analysis across 19 bee species, including *A. mellifera*, showed high variability in LD_{50} values among species (Arena & Sgolastra, 2014). In contrast to most wild bees, honey bees have a larger body-size, construct nests with resin and wax, are more broadly polylectic (resource generalist), and have a much larger foraging range. They are also advanced eusocial organisms with colony sizes of more than ten thousand individuals. These different life-history traits could mean

that honey bees could encounter very different levels of pesticide risk than wild bees, even in the same locations (Brittain & Potts, 2011; Kopit & Pitts-Singer, 2018; Sgolastra et al., 2018a). Indeed, body size differences can alter bee surface to volume ratios and change pesticide contact absorption rates (Valdovinos-Núñez et al., 2009), while collection of different nesting substrates can result in different routes of pesticide exposure (Kopit & Pitts-Singer, 2018). Differences in floral diet preferences between wild bees and honey bees could greatly influence the levels of pesticide risk they encounter. For example, some solitary bees have evolved to prefer or even specialize exclusively on crop pollen (see Gibbs, Elle, Bobiwash, Haapalainen, & Isaacs, 2016; López-Uribe, Cane, Minckley, & Danforth, 2016; Minckley, Wcislo, Yanega, & Buchmann, 1994), which could greatly increase their risk of pesticide exposure compared with polylectic honey bees (Brittain & Potts, 2011). Foraging range could also play a major role in differential pesticide risk between wild and managed bees. For instance, wild bees nesting near a crop might be forced to interact with crop pesticides more than honey bees because of their smaller foraging range. In contrast, honey bees could potentially forage on uncontaminated floral resources by flying farther afield.

In fact, two recent studies have shown that honey bees are not an adequate model of wild bee performance in agroecosystems. In oilseed rape, wild bees showed reduced density and nesting rates in fields treated with neonicotinoids, while honey bees did not respond to pesticide treatment (Rundlöf et al., 2015). Also in oilseed rape, wild bees produced fewer nest cells as neonicotinoid exposure in their nests increased, while honey bee response to pesticide use during oilseed flowering varied depending on the countries where experiments were conducted (Woodcock et al., 2017). Thus, while species *performance* has been shown to differ in response to pesticides, we are still lacking research that directly compares *pesticide risk levels* between wild bees and honey bees in the same crops.

Here, we ask if honey bees (*A. mellifera*) and wild bees (*Osmia cornifrons*) experience similar levels of pesticide exposure and risk (agrochemical exposure in ppb *in terms* of toxicity or LD_{50}) in the

same 14 apple orchards during bloom, and we explore whether risk levels are influenced by cropland cover, crop pollen collection, and crop foraging radius. We chose to compare *A. mellifera* with *O. cornifrons* because *O. cornifrons* has an established preference for rosaceous pollen resources, including apple (Batra, 1998; Haider, Dorn, Sedivy, & Müller, 2014). *Osmia cornifrons* also has been shown to collect pollen from only nine plant families (Haider et al., 2014), constituting a much narrower diet breadth than *A. mellifera*, which has been shown to collect pollen from 41 to 80 plant species, in 25 different families, in just one season (Bilisik & Cakmak, 2008; Ismail, Owayss, Mohanny, & Salem, 2013; Synge, 1947). In addition, *O. cornifrons* is thought to forage at much smaller radii than *A. mellifera*, based on their body size (Guédot, Bosch, & Kemp, 2009) and homing range (Kitamura & Maeta, 1969). Though maximum foraging distance has not been determined for *O. cornifrons*, distances range between 475 and 1000 m across four *Osmia* species (Gathmann & Tscharntke, 2002), while *A. mellifera* has been shown to have a mean foraging distance of 5.5 km (Beekman & Ratnieks, 2000).

We predict the following:

1. For both species, increasing apple land cover will lead to increased pesticide risk levels in bee-collected pollen, *via* increased proportion apple pollen collected (see Fig. 3.1 for a graphical representation of our prediction).
2. Compared with *A. mellifera*, *O. cornifrons* will collect a higher proportion apple pollen and will encounter higher levels of pesticide exposure and pesticide risk in their pollen, given their innate preference for Rosaceous pollen and their smaller foraging radius.

Methods:

Study System and Sampling:

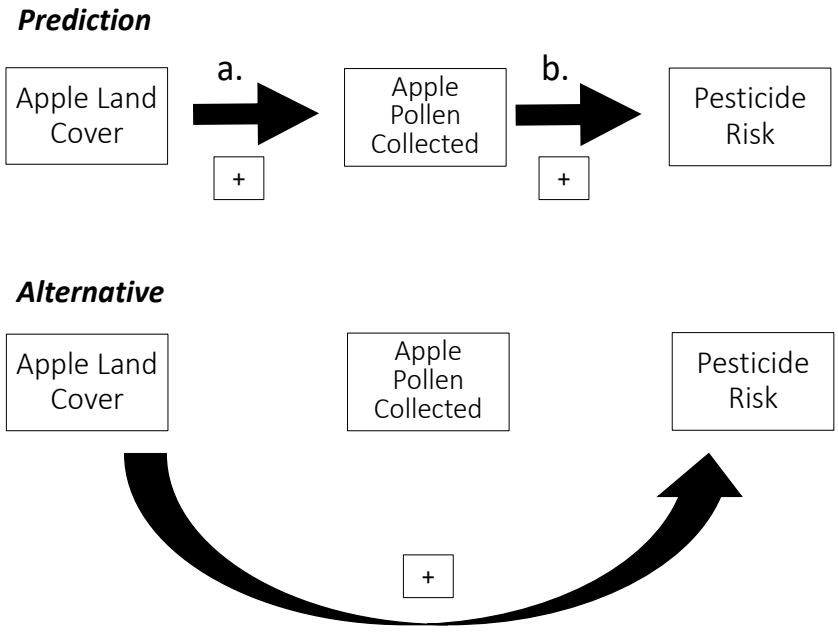

Figure 3.1. The predicted indirect positive effect of apple land cover on pesticide risk levels in bee-collected pollen (upper path; see predictions in Introduction). We expect that, as apple land cover increases, bees will collect a greater proportion apple pollen (a), which, in turn will lead to higher pesticide risk levels in their pollen provisions and beebread (b). The alternative hypothesis (lower path) is that increasing apple land cover will result in higher pesticide risk levels in bee-collected pollen, but that this effect will not be mediated by the proportion apple pollen collected.

Our goal was to compare pesticide risk levels in bee-collected pollen between the model bee *A. mellifera* (here after "*Apis*"), and wild bees. We chose *O. cornifrons* (here after "*Osmia*") to represent wild bees. Similar to many wild bee species, *Osmia* bees are solitary, univoltine, polylectic, and mass-provisioning (Bosch & Kemp, 2001). In addition, female *Osmia* nest above-ground in trap-nests which makes it possible to easily collect their pollen provisions to assess pollen host plant use and pesticide risk levels. We chose apple as our cropping system because *O. cornifrons* is known to pollinate apples (Maeta, 1990), and apple is an economically important crop in upstate New York. In addition, upstate New York apple orchards are relatively small, so we were more likely to capture the differential effects of foraging distance between species. Indeed, the 14 focal apple orchards we chose were all contained by a 600 m or smaller radius about about bee nests and hives, and maximum homing distance, at which 50% of displaced females returned to their nest, was estimated at 500 m for *O. cornifrons*, such that we would expect *O. cornifrons* to forage mostly in the orchards, while *A. mellifera* forages at much larger distances (Beekman & Ratnieks, 2000; Kitamura & Maeta, 1969).The 14 focal orchards selected (Fig. A3.1) ranged in both size (0.81 to 58.5 ha) and pesticide management. Spray frequency per site during our 32-day study period ranged from 0 (at an abandoned orchard) to 39 (at a conventional orchard) sprays.

Apple Orchard Land Cover and Bloom:

To determine the foraging radius that best predicted the proportion *Malus* (a proxy for apple, see below) pollen collected by each species, we measured the proportion apple land cover surrounding bee nests and hives at six radii (250, 500, 1000, 1500, 2000, 3000 m) and chose the most predictive radius (see Statistics section) as the "*Malus* foraging radius" for both species. While these selections are likely associated with the radius at which bees collected *Malus* pollen specifically in our study, it is important to note that they may not be predictive of the actual foraging range of each bee on other

resources or in other contexts outside of our study.We chose these six candidate radii because the maximum homing range, at which 50% of displaced foragers returned to nests, has been estimated at 500 m for *O. cornifrons*, and 3 km for *A. mellifera* (Kitamura & Maeta, 1969; Pahl, Zhu, Tautz, & Zhang, 2011). In addition, previous research demonstrated that, depending on the land cover habitat measured, pollen foraging in apple is best predicted between 250 and 1250 m surrounding *O. cornifrons* nests (Centrella et al., in prep) and at a 3 km radius surrounding *A. mellifera* hives (McArt et al., 2017). We used ArcGIS [ArcMap 10.5.1] and the USDA 2015 Crop-Scape Data Layer to quantify the proportion apple land cover surrounding nests and hives at the six different radii. Orchard area was measured by overlaying polygons (Fig. A3.1) on farm perimeters in the Google Satellite layer in QGIS version 3.4. In some cases, orchard area estimates were higher than the apple land cover estimated by the Crop-Scape data layer. In these cases, we used our ground-truth estimates from QGIS instead of the Crop-Scape estimates.

We confined our study to apple bloom, so that we could accurately measure how crop pollen collection related to pesticide exposure and risk. Thus, bloom was carefully monitored at every sampling date (see Table A3.1), and sites with petals present on apple trees were considered “in bloom”. Bloom start times were recorded as the first sampling date at a site where bloom was observed. If bloom had already started at sites upon the first sampling date, bloom start date was listed as “prior”. Bloom end date was recorded as the date of sampling where petal fall had reached completion (Table A3.1).

Bee Establishment and Sampling:

We purchased 56, 5-frame nucleus colonies of *A. mellifera* from a local commercial beekeeper in April of 2015. Colonies were transferred to new, 10-frame equipment with plastic foundations and, for two weeks, bees were left to draw comb in a common location (Dyce Lab for Honey Bee Studies, Ithaca, NY: 42.466118, –76.446211). At this time, we assessed colony strength and queen status, and installed

new queens from the same genetic source where necessary. During this time, we assessed each colony's composition and redistributed frames among colonies to ensure that all had a similar composition of brood, bees, pollen and honey before transferring them to the field experiment sites. Concurrent with apple bloom, we transferred four honey bee colonies to each of the 14 apple orchard sites during May 7-11.

Prior to the experiment, in 2014, *Osmia* experimental source populations were pre-established at six suburban backyards within the town of Ithaca, NY (longitude: 42.428527 to 42.469463, latitude: -76.530422 to -76.465609 DD). We used nest tubes that were 15.24 cm in length by 7.5 mm inner diameter, with paper inserts, to assist pollen provision removal [crownbees.com]. During the fall preceding the experiment, we used x-radiography at 52 kVp and 3.2 mAs to measure overwintering bee number to ensure that nests were healthy (i.e. containing no dead larvae or nest parasites). Just prior to the start of apple bloom (May 5, 2015), wooden nest shelters at field sites were randomly assigned 30 to 32 source population nest tubes containing 98 to 102 overwintering bees. Nest tubes containing healthy bees were assigned random numbers so that there was a roughly equal representation of the six source sites at each experimental nest site, ensuring equivalent genetic variation across the experimental populations. Source tubes were marked so we would not confuse them with newly completed experimental nest tubes. *Osmia* were placed in orchards on May 4 through 5, so that their emergence roughly coincided with honey bee placement (May 7 through 11) and with apple bloom (May 4 through May 30; see Table A3.1).

Bees were left to forage until apple bloom ceased. We sampled *Apis* hives twice (May 15 through 19, and June 1 through 5) and *Osmia* nests three times (from initial nest completion on May 20 to end of bee activity on June 6). Though both species completely overlapped with each other at all sites, we sampled *Apis* on different dates than *Osmia*. Thus, to maximize comparability between species, we only selected sampling dates where species were sampled within six days of one another at a site. In

addition, because apple bloom time varied depending on the site, we only selected sampling dates within six days of bloom at each site. We chose a six day interval because *Osmia* females take about one week to complete nests (Bosch & Kemp, 2001), and *A. mellifera* larval development and beebread consumption can last up to six or seven days (Aupinel et al., 2005). Thus, pollen collected from nests tubes and hives six days after bloom could still contain pollen that was collected by bees during bloom. Following the above criteria, sampling date selection resulted in 17 *Apis* sample dates and 22 *Osmia* sample dates (see Table A3.1). We took the site average across these selected sampling dates, resulting in one observation per site, in order to directly compare the two species.

Pollen Collection:

For *Osmia*, completed nest tubes (with an apical mud cap) were collected during each sampling date and nest tube number varied depending on bee activity (see Table A3.1). Nest tubes were frozen at -20 ^{o}C within 5 hours of collection to preserve pesticide residues. Assessment of pollen diet and pesticide exposure were averaged across all nest tubes collected per sampling date per site. For *Apis*, at each sampling date, we collected approximately 3 g recently accumulated beebread from newly drawn comb, so that pesticide residues and pollen diet analyses were more closely associated with each sampling date. At every sampling date, we assessed queen status, the presence of swarm and supercedure cells, and the hive area occupied by bees, to determine swarm likelihood. Assessment of pesticide exposure and pollen diet were averaged across four hives at each site per sampling date, except for six hives that were removed from the analysis because they swarmed within a week of a sampling date (see "Hive" column in Table A3.1). Beebread was immediately placed on dry ice and stored in a -80 ^{o}C freezer until analysis. To assess pollen diet and pesticide exposure, we homogenized pollen provisions and beebread collected from each sampling date. *Osmia* pollen provisions were homogenized in equal amounts (within 0.05 g) across nest tubes, so each adult female had equal

representation. Similarly, the ~3 g of *Apis* beebread collected per hive was homogenized in equal ratios per sampling date per site.

Pollen Diet Assessment:

For each sampling date, 24-25 mg of homogenate pollen was combined with 200 µl of water for *Osmia* or 500 µl of water for *Apis,* due to different pollen consistencies. Pollen homogenates for both species were then vortexed for 15 s, and sonicated for 2 min to break up chunks of pollen. Ten µl of the resulting solution was pipetted onto microscope slides with 38-40 µl of Calberla's stain solution. Using an Olympus BX41 compound light microscope at 40x magnification, we counted 300 pollen grains per slide within randomly-generated field-of-view transect(s), excluding obviously broken exines and grains that were not fully within the transect (Laura Russo & Danforth, 2017). Pollen grain morphotypes comprising greater than 3% per sample (i.e. exceeding the threshold for accidental contact) were identified to family, except for Rosaceae morphotypes, which were identified to genus where possible. We recognized an "Other" type within Rosaceae for those which could not be identified to genus. Two of the *Apis* pollen types ("Fabaceae/Apiaceae" and "Unknown") could not be resolved to family. Identification hypotheses were established using pollen keys and image libraries (Girard, 2014; Kapp, Davis, & King, 2000; Laura Russo, 2014), floral ranges and bloom times (USDA, 2017; Weldy, Werier, Nelson, Landry, & Campbell, 2017), and voucher specimens from the same sites that were collected in 2014 (Russo, unpublished). All *Apis* samples were further verified by P. Lau and V. Bryant. Because we could not distinguish pollen types to species, pollen identified to the genus *Malus* may have contained wild crab-apple species, but *Malus* pollen is most likely from cultivated apple, as crab-apple surrounding experimental sites was noted as scarce.

Pesticide Quantification and Risk Assessment:

We tested bee pollen homogenates for 33 pesticide active ingredients, including 18 fungicides and 15 insecticides. Twenty-five of the active ingredients were commonly used, exceeding the yearly median pounds sprayed for active ingredients across New York State in 2013 (Pesticide Sales Use Reporting, 2013). Also, 12 of the active ingredients tested represented 43% of those sprayed by growers in our focal orchards during the study period. For both species, we extracted and purified 1 to 3 g of homogenate pollen per sampling date via a modified version of the QuEChERS protocol (Lehotay, De Kok, Hiemstra, & Van Bodegraven, 2005), then analyzed the samples on a LC-MS/MS system. For both species, homogenates were combined with water, acetonitrile, an internal imidacloprid standard, and an extraction buffer (magnesium sulfate and sodium acetate). Then a dispersive solid phase extraction (d-SPE) clean-up was performed using $MgSO_4$-PSA and C-18 silica. Extracts were then gradient-eluted in water and acetonitrile/water for *Apis,* and in water and methanol for *Osmia.* For details, see Stoner & Eitzer for *Osmia* (Stoner & Eitzer, 2013) and Appendix A3.1, in Appendix 3, for *Apis.* Residues were analyzed using a LC-MS/MS system [Agilent 1200 Rapid Resolution LC-MS coupled with a Thermo-LQT linear ion trap Mass Spectrometer] equipped with a C18 column [Zorbax SB-C18 Rapid Resolution HT 1.8 µm, 2.1 x 50 mm] for *Osmia*, and a LC-ESI-MS/MS system [Vanquish UHPLC coupled with a TSQ Quantis Mass Spectrometer; Thermo Scientific] equipped with a C18 reversed-phase column [Accucore aQ 2.6 µm, 100 x 2.10 mm; Thermo Scientific] for *Apis.* Details on analyte concentrations and quality assurance can be found in Stoner & Eitzer (Stoner & Eitzer, 2013) for *Osmia* and Appendix A3.1 for *Apis*, as well as in Table 3.1 and Table A3.2 for both species. Active ingredients found in amounts below the limit of quantification (LOQ) were summed per sampling date, and then the average of the sampling date sums was taken, to estimate pesticide exposure (in ppb) at the site-level.

To estimate pesticide risk in bee-collected pollen, we used the pollen hazard quotient (HQ), which expresses exposure (ppb) *in terms of* toxicity (bee LD_{50}) per active ingredient (Stoner & Eitzer, 2013). We calculated per site HQ by taking the summation of each active ingredient's HQ (see below):

Table 3.1. Pesticide detection information, exposure, and risk for the 19 active ingredients detected across sites in *Apis* beebread and *Osmia* pollen provisions. Chemicals are listed in alphabetical order, along with their type (fungicide or insecticide) and whether they were were reportedly sprayed by growers in the apple orchards coinciding with our study. The acute, 48-hour, topical LD_{50} value (in μg/g) used for the risk assessment is listed for each chemical. LD_{50} values that were not exact in the literature are indicated in parentheses next to the selected LD_{50} value (see Methods). For each active ingredient, the site-level average exposure (ppb) as well as the average exposure where "found" (detected above the limit of quanitification) is listed. For each active ingredient, the average risk level (% hazard quotient, or %HQ) across time points is shown, as well as their percent contribution to the total summed risk (%HQ) across all time points. The average limit of quantification (LOQ) across sites is shown in ppb, along with the number of sites (of 14 total sites) where each chemical was found (above the LOQ). LOQ values of the 14 active ingredients that were tested and *not* detected can be found in Appendix 3 (Table A3.2). "QLNT" stands for Quantitation Limit Not Tested. See Table A3.2 legend for details on LOQ estimation for each species.

				Apis					*Osmia*				
active ingredient	sprayed in focal orchards	type	topical LD_{50} (micrograms/bee)	ave exposure (ave where found) (ppb)	average risk (%HQ)	% of total risk	average LOQ (ppb)	# (of 14) sites where found	ave exposure (ave where found)(ppb)	average risk (%HQ)	% of total risk	average LOQ (ppb)	# (of 14) sites where found
acetamiprid	yes	insecticide	8.09	0.519 (3.373)	0.001	0.005	0.42	2	3.714 (52.000)	0.009	0.028	1.67	1
carbaryl	yes	insecticide	0.14	20.470 (33.264)	1.974	11.282	1.05	8	205.321 (410.643)	27.278	89.212	2.33	7
chlorpyrifos	no	insecticide	0.059	35.769 (464.998)	8.184	46.779	140	1	2.593 (18.150)	0.817	2.673	2	2
clothianidin	no	insecticide	0.00368	not found	0.000	0.000	35	0	0.237 (1.658)	1.197	3.916	1.33	2
cyprodinil	no	fungicide	785 (>784)	53.987 (70.184)	0.001	0.005	0.7	10	140.735(218.920)	0.003	0.011	10	9
difenconazole	yes	fungicide	178 (>177)	17.843 (28.994)	0.001	0.008	1.05	8	47.181 (82.567)	0.005	0.016	0.83	8
fenbuconazole	yes	fungicide	293 (>292)	6.711 (87.249)	0.000	0.002	14	1	2.819 (19.733)	0.000	0.001	2	2
fenpyroximate	no	insecticide	15.8	0.0560 (0.387)	0.000	0.000	0.21	2	not found	0.000	0.000	QLNT	0
fluxapyroxad	yes	fungicide	445 (>444)	4.858 (21.052)	0.000	0.001	14	3	120.976 (564.556)	0.005	0.017	QLNT	3
imidacloprid	no	insecticide	0.0038	not found	0.000	0.000	3.5	0	0.236 (3.300)	1.154	3.773	QLNT	1
indoxacarb	yes	insecticide	0.068	32.671 (424.719)	6.486	37.072	11.2	1	not found	0.000	0.000	1	0
phosmet	yes	insecticide	0.22	8.800 (114.398)	0.540	3.086	11.2	1	not found	0.000	0.000	2	0
pyraclostrobin	yes	fungicide	101 (>101)	6.9573 (15.075)	0.001	0.005	0.28	6	98.742 (460.794)	0.018	0.059	QLNT	3
pyrimethanil	no	fungicide	101 (>100)	10.235 (22.176)	0.001	0.008	0.35	6	15.143 (106.000)	0.003	0.009	6	2
spinetoram_l	yes	insecticide	0.011	0.028 (0.364)	0.034	0.196	0.35	1	not found	0.000	0.000	QLNT	0
thiacloprid	yes	insecticide	17.94	not found	0.000	0.000	0.28	0	0.993 (4.633)	0.001	0.003	1	3
thiamethoxam	yes	insecticide	0.024	0.480 (6.238)	0.270	1.543	1.05	1	0.099 (0.692)	0.077	0.250	1	2
thiophanate-methyl	no	fungicide	101 (>100)	0.417 (5.426)	0.000	0.000	0.14	1	9.929 (139.000)	0.002	0.006	QLNT	1
trifloxystrobin	no	fungicide	201 (>200)	18.045 (29.323)	0.001	0.007	0.14	8	82.954 (290.338)	0.008	0.025	0.67	4

$$HQ\ per\ site = \Sigma \frac{active\ ingredient\ in\ ppb\left(\frac{\mu g}{kg}\right)}{LD50\left(\frac{\mu g}{bee}\right)}$$

Next, to express HQ in terms of risk to an individual bee, we had to account for the *amount* of pollen a bee is exposed to. We modified the HQ measurement, multiplying by the pollen exposure (grams of pollen consumed) per bee. We then divided by 1000 to simplify HQ in terms of proportion of the LD_{50}, and then multiplied by 100, so that %HQ was in terms of $\%LD_{50}$, as shown below:

$$\%\ Hazard\ Quotient\ per\ bee\ per\ site = \Sigma \frac{active\ ingredient\left(\frac{\mu g}{kg}\right) * \frac{pollen\ consumed\ (g)}{larval\ bee}}{LD50\left(\frac{\mu g}{bee}\right) * 1000} * 100$$

We estimated pollen exposure to bees in terms of larva consumption because this is the most direct route of pollen exposure to bees and because larval pollen consumption is the most accurate comparison of exposure between species. Both species are enclosed as larvae, and both spend up to six days consuming pollen provisions and bee bread (Aupinel et al., 2005; Bosch & Kemp, 2001). Ideally, we would have used species-specific oral, larval LD_{50} values to estimate toxicity, but LD_{50} values for *O. cornifrons* were scarce at the time of our study and oral and larval LD_{50} values were not available for all compounds in our study, so we used adult 48 hr contact LD_{50} values for *A. mellifera* to estimate toxicity to both species. Previous use of this metric shows that it is biologically relevant, as it predicted *O. cornifrons* offspring number and weight (Centrella et al., submitted). However, it is important to note that our risk estimates are unlikely to directly translate to mortality rates, as we are dividing *oral larval* pollen consumption by *adult contact* LD_{50}s.

To estimate larval pollen consumption for *Osmia*, we calculated the average weight of 994 pollen provision masses using a Mettler Toledo AG245 Analytic Laboratory Scale. Pollen provisions averaged 186 ±4 mg, so we used 186 mg for the %HQ calculation. For *Apis*, we used 135 mg, or the average of two literature estimates (125 and 145 mg) of the mean amount of beebread necessary to

raise a single worker larva (Alfonsus, 1933; Rosov, 1944). To emulate a worst-case scenario, we assumed that larvae consumed their entire pollen provisions over 48 hours, and we used the lowest available 48-hour, contact LD_{50} values from three sources (EPA, 2018; IUPAC, 2017; Tomlin, 2009). In cases where exact values were not available and toxicity was reported to be "greater than" a value, we conservatively assumed the LD_{50} value to be the next highest whole number of the highest number listed. We confined our analysis to insecticides and fungicides. Insecticides, designed to affect insects, are directly toxic to bees. However, fungicides also have been shown to directly reduce bee reproduction (Bernauer et al., 2015) and foraging and nesting behavior (Ladurner et al., 2008), as well as synergize with insecticides to exacerbate their toxicity to bees (see Sgolastra et al., 2016; 2018b; Raimets et al., 2017). In contrast, the direct and indirect impacts of herbicides on bees are largely unexplored (but see Motta et al., 2018). Due to lack of beebread at one site, pesticide risk and exposure for *Apis* could only be assessed at 13 of our 14 sites (see Table A3.1). Thus, all comparisons of pesticide exposure and risk between species were based on 13 observations.

To determine the amount of pesticide exposure and risk that could be attributed to apple management, we assessed the active ingredients of products reported in grower spray records at the 14 sites. Active ingredients were considered "apple sprays" if they were reportedly sprayed by growers starting with the earliest spray of the season at our orchards (May 6), or two days after initial bee placement in the orchards (May 4), through the date of our final sampling at each site (Table 3.1). Active ingredients not reportedly sprayed by growers were considered "other sprays", which were likely applied outside of the focal orchards. While we can be sure that "other sprays" were not applied in our orchards during bee activity, our presence-absence analysis likely over-estimated the "apple sprays". Indeed, the active ingredients reportedly sprayed (Table 3.1) are not restricted to apple products and sprays are subject to drift from adjacent crops (Woods, Craig, Dorr, & Young, 2001).

Statistics:

All analyses were conducted using the computer program R, version 3.5.1 (R Core Team, 2018). In order to test whether *Osmia* collected a greater proportion *Malus* pollen (n=14), higher pesticide exposure levels (n=13), and higher pesticide risk levels (n=13) than *Apis*, we averaged these variables by site and subtracted *Apis* means from *Osmia* means. We then conducted single-distribution, one-sided t-tests or Wilcoxon Signed-Rank tests to ask whether *Osmia* means were greater. For the difference in pesticide risk, we used a t-test. However, because the species differences for proportion *Malus* pollen and pesticide exposure data were not normally distributed, we conducted non-parametric Wilcoxon Signed-Rank tests with continuity corrections, in place of t-tests. The t-test and Wilcoxon tests were conducted in base R, using the functions t.test and wilcox.test (R Core Team, 2018). In order to ensure that our species comparisons were not significantly influenced by zeros, for each species we summed the number of sites with and without zeros for proportion *Malus* pollen, pesticide exposure, and pesticide risk. We then conducted three X^2 tests for independence using the chi.square function in base R, in order to test whether null values were independent of species at the site level.

In order to choose the apple land cover radius that best reflected the foraging radius at which *Malus* pollen was collected, we created six single-predictor linear models (n=13) using the lm function in the lme4 package in R (Bates, Mächler, Bolker, & Walker, 2015). Model predictors were the proportion apple land cover at a 250, 500, 1000, 1500, 2000, and 3000 m radius, and the model response (for all models) was the proportion *Malus* pollen collected. Because the response was proportional, it was log (plus 0.01) transformed. Otherwise, for variable selection purposes, we assumed linear and normal model distributions. To select the most predictive apple land cover radius, we ranked models by the log-likelihood Akaike Information Criterion (AIC) value (Burnham & Anderson, 2002) using the AIC function in base R, and selected the lowest-ranking models to represent *Malus* pollen "foraging radius" for each species.

Path analysis:

To assess our predicted (see Fig. 3.1) direct and indirect relationships between the proportion apple land cover, proportion *Malus* pollen collected, and pesticide risk, we used piece-wise structural equation (SEM) models (Shipley, 2009), which allow variables to simultaneously act as predictors and responses. We designed our path analysis to test if the proportion apple land cover influenced pesticide risk to bees, mediated by the proportion *Malus* pollen in pollen provisions and beebread. In this case, the proportion *Malus* pollen acted as both a predictor and a response (see Fig. 3.1). Path analysis models were constructed using the lm function in the lme4 package in R (Bates et al., 2015). For both species, paths were composed of the following models:

a. proportion *Malus* pollen as the response, with the proportion apple land cover within bee foraging radius as the predictor (Fig. 3.1, arrow a)

b. pesticide risk as the response, with proportion *Malus* pollen as the predictor (Fig. 3.1, arrow b)

Path model residuals were graphically inspected to ensure that there were no violations of normality and homoscedasticity. For both paths, proportion *Malus* pollen was log (plus 0.01) transformed. For the *Apis* path, pesticide risk data were positively skewed and thus, log (plus 0.01) transformed. Transformed path models were tested for spatial autocorrelation ($-0.141 < r < 0.067$, $p > 0.467$) in the residuals using the Mantel test (ade4 package, Dray & Dufour, 2007). Model variables were visually inspected for potential outliers, and, for *Osmia*, a potential high outlier was identified in the distribution of the proportion apple land cover at a 1 km radius. We used Grubbs' single outlier test in the outliers package in R (Komsta, 2011) to determine statistical significance of this outlier. The overall fit of the two path models was assessed by testing the proposed path model relationships against the independence claim, which, in this case, is the direct effect of the proportion apple land cover on pesticide risk (Fig. 3.1, alternative path) using Shipley's d-separation test, which compares observed correlations to random variation using the Fisher's chi-square C-statistic (Shipley, 2009). Statistics for

path models were calculated and fitted by maximum likelihood methods using the piecewiseSEM package in R (Lefcheck, 2017). Correlation coefficients were standardized by mean and standard deviation. Path analysis models were based on 14 observations for *Osmia* and 13 observations for *Apis*, due to lack of pesticide data at one site (see *Pesticide Risk Assessment*).

Results:

Twenty floral types were collected across species: ten types from eight families for *Osmia*, and 14 types from nine families for *Apis* (Fig. 3.2A&B). The plant family collected with the highest mean proportion was Caprifoliaceae (0.410 ± 0.086 se) for *Osmia* and Rhamnaceae (0.518 ± 0.070 se) for *Apis* (Fig. 3.2A&B). Nineteen pesticide active ingredients were detected in bee-collected pollen across sites and species, 11 of which were reportedly sprayed by apple growers. The active ingredients with the highest contribution to %HQ were carbaryl and chlorpyrifos, for *Osmia* and *Apis*, respectively (Table 3.1). Over 80% of insecticide concentrations detected in bee-collected pollen could be attributed to active ingredients reportedly sprayed in apple orchards (Fig. 3.3A), for both *Osmia* (0.986 ± 0.008 se, n=7) and *Apis* (0.805 ± 0.114 se, n=12). In contrast, only around half of fungicide concentrations detected in bee-collected pollen resulted from active ingredients used in apple production (Fig. 3.3A), for both *Osmia* (0.490 ± 0.112 se, n=11) and *Apis* (0.429 ± 0.102 se, n=11). For both species, the %HQ was driven almost entirely by insecticides (Fig. 3.3B), even though fungicide exposure was found at similar exposure levels (Fig. 3.3A); this is due to the fact that insecticides were more toxic (LD_{50} <18 μg/bee) than fungicides (LD_{50} > 100 μg/bee; see Table 3.1). Similar to insecticide exposure, insecticide risk could be mostly attributed (Fig. 3.3B) to apple active ingredients for both *Osmia* (0.913 ± 0.023, n=7) and *Apis* (0.821 ± 0.120 se, n=11).

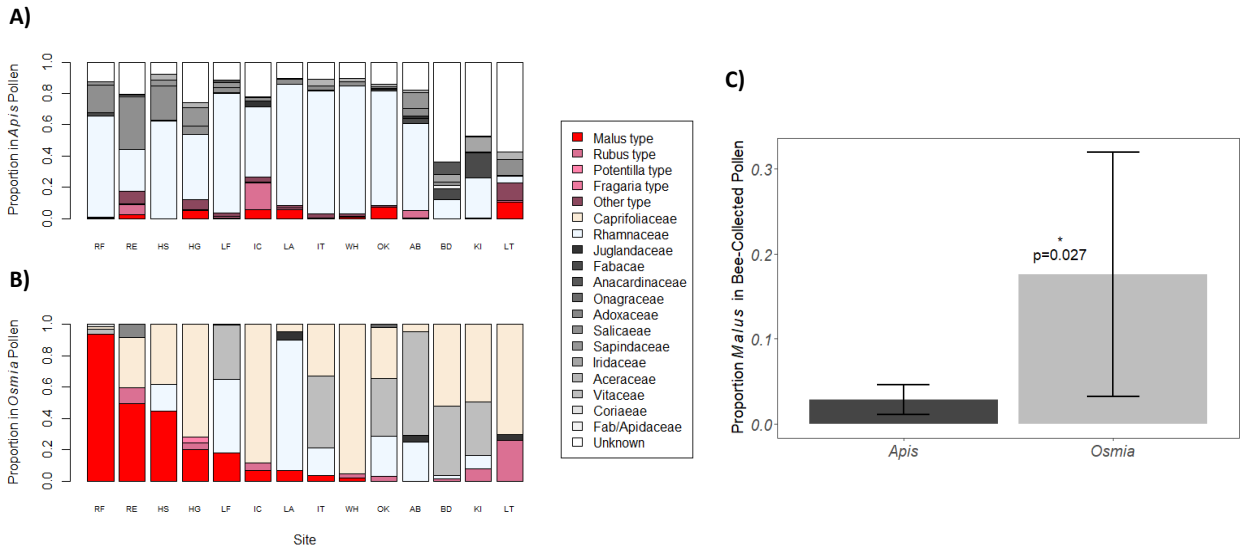

Figure 3.2. Mean proportion of pollen types collected per site for both *Apis* (A) and *Osmia* (B), in descending order of proportion *Malus* pollen (shown in red) collected by *Osmia*. Twenty floral types and 14 floral families were collected by the two species across sites, with *Osmia* collecting ten floral types (eight distinct families), and *Apis* collecting 14 types, (nine families). Rosaceous pollen types are shown in reds and pinks while the most-collected resource (see Results) for *Osmi*a, Caprifoliaceae, is shown in beige and for *Apis*, Rhamnaceae, is shown in blue. Across all 14 sites, *Osmia* (grey bar, C) collected a higher mean proportion *Malus* pollen than did *Apis* (black bar, C). Lines super-imposed on bars show the 95% confidence intervals (±1.96 SE) for each mean.

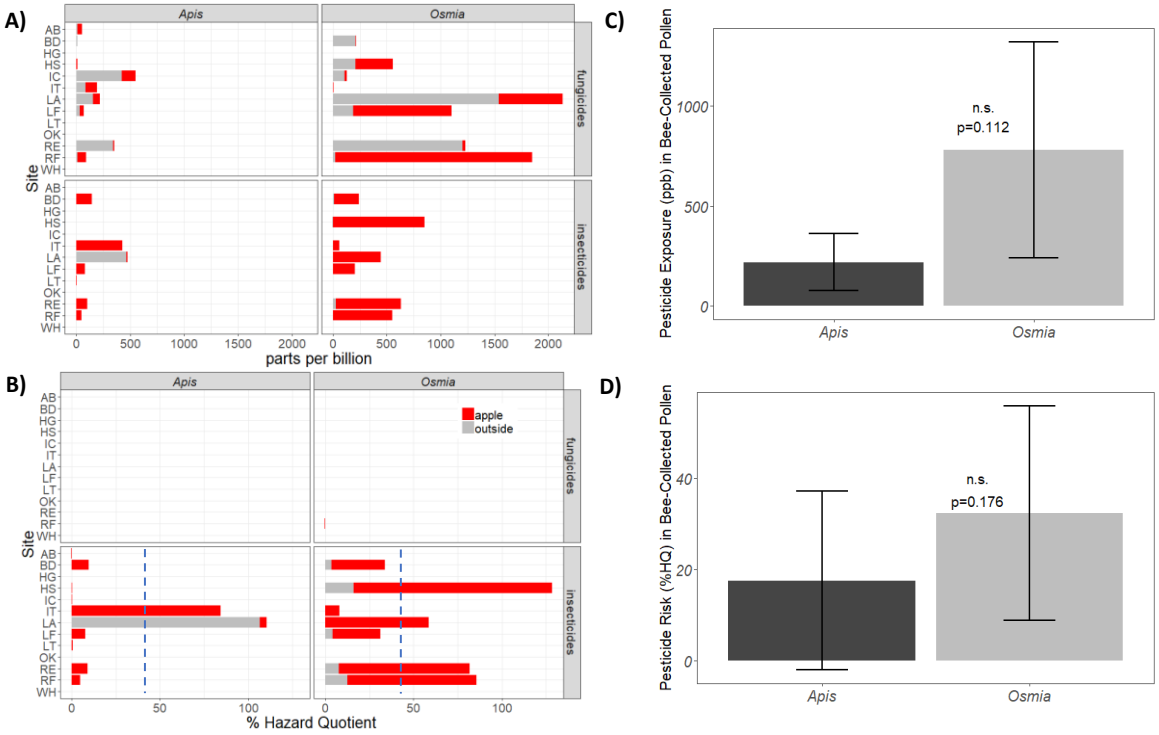

Figure 3.3. Average pesticide exposure in ppb (A) and pesticide risk in % Hazard Quotient (B) detected in *Apis* beebread (left panels) and *Osmia* pollen provisions (right panels) attributed to both fungicides (upper panels) and insecticides (lower panels) across the 13 apple orchard sites for which data for both species were available. Bright red portions of bars represent pesticide active ingredients reportedly sprayed by apple growers during bee activity at each site, while grey portions represent active ingredients applied outside of focal orchards. Dashed blue lines (B) represents the United States Environmental Protection Agency Tier 1 level of concern for acute contact pesticide risk (US EPA, 2014). Across sites, mean pesticide exposure (C) and pesticide risk (D) in bee-collected pollen was not significantly greater for *Osmia* (light grey bars) compared with *Apis* (dark grey bars). Lines super-imposed on bars represent the 95% confidence intervals for each mean (± SE * 1.96).

As expected, the most predictive apple land cover radius at which bees collected *Malus* pollen (see Table A3.3) was smaller (1 km) for *Osmia* when compared to *Apis* (3 km). At these scales, the maximum proportion apple land cover, and likely apple flower availability, was as high as 0.18 per site for *Osmia*, but did not exceed 0.021 for *Apis* (Fig. A3.1).

Relationship between apple land cover, apple pollen, and pesticide risk levels (Prediction 1):

In accordance with our prediction, *Osmia* collected a higher proportion *Malus* pollen as apple land cover within their foraging radius (1 km) increased (Fig. 3.4, Fig. 3.5A, Table 3.2), and this increase in the proportion *Malus* pollen resulted in increased levels of pesticide risk in *Osmia* pollen provisions (Fig. 3.4, Fig. 3.5B, Table 3.2). This result suggests that increasing proportion apple land cover indirectly correlated with increased pesticide risk (R^2=0.52), via higher proportions of *Malus* (likely apple) pollen collected (Fig. 3.4, Fig. 3.5A&B, Table 3.2). Our analysis with proportion apple land cover at 1 km was influenced by a statistically significant (G=2.92, U=0.29, p=0.001) outlier, so further study encompassing sites representing a wider range of surrounding apple land cover is necessary to confirm these results.

Interestingly, there was no relationship between the proportion apple land cover at 3 km and the proportion *Malus* pollen collected by *Apis* (Fig. 3.5C), nor was there a relationship between the proportion *Malus* pollen and pesticide risk levels in beebread (Fig. 3.5D). Although pesticide risk levels in beebread were poorly explained by our path model (R^2=0.06), our data were more consistent with the path model than with the alternative hypothesis (Fig. 3.1), suggesting that there is no direct impact of proportion apple land cover on pesticide risk in *Apis* beebread (Fig. 3.4, Fig. 3.5C&D, Table 3.2).

Species comparison (Prediction 2):

Supporting our prediction, *Osmia* collected a greater mean proportion *Malus* pollen than did *Apis*, (V=64, p=0.027, n=14; Fig. 3.2). On average across sites, *Osmia* collected over 6 times the proportion *Malus* pollen (mean 0.176 ± 0.073 se) compared to the proportion collected by *Apis* (0.029 ±

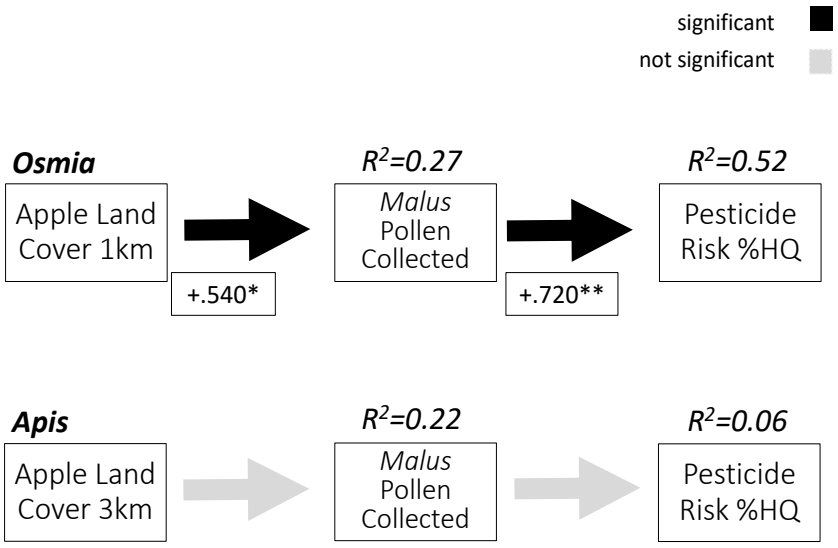

Figure 3.4. Resulting path models for *Osmia* (upper path; Fisher's C=1.026, p=0.599, df=2, n=14) and *Apis* (lower path; Fisher's C=0.96, p=0.619, df=2, n=13). Proportion apple orchard land cover (within the *Malus* pollen foraging radius of each species) indirectly correlated with increased pesticide risk levels in *Osmia* pollen, via an increased proportion *Malus* pollen collected in pollen provisions. These relationships were not significant for *Apis*. Unidirectional arrows represent relationships (black significant, grey not significant) between variables (in boxes). Standardized correlation coefficients are shown in boxes alongside arrows accompanying p-value significance levels (0.01<p<0.05=*, 0.001<p<0.01=**) and R^2 values are shown above estimated variables. Statistics are based on transformed variables (see Table 3.2).

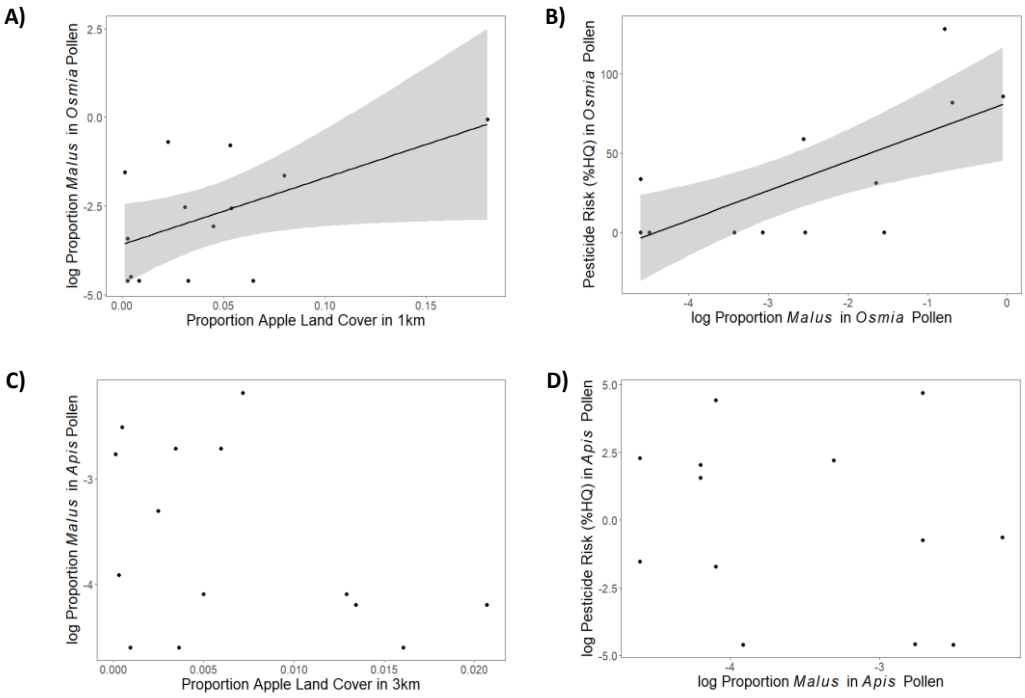

Figure 3.5. The pairwise relationships found in the path analyses for both *Osmia* (A,B) and *Apis* (C,D). For *Osmia*, the log proportion *Malus* pollen collected increased with increasing apple orchard land cover (A; p=0.0464, n=14), and, as the log proportion *Malus* increased in pollen provisions, pesticide risk also increased (B; p=0.0037, n=14).For *Apis*, there was no relationship between the log transformed proportion *Malus* pollen collected and the proportion apple land cover at 3 km surrounding hives (A; p=0.106, n=13), and also no relationship between the log proportion *Malus* pollen collected and log pesticide risk (% hazard quotient) in *Apis* bee bread (D; p=0.413, n=13). Points show site observations, lines denote relationships between variables, and grey shadows represent 95% confidence intervals (Statistics here are based on multi-modal SEM models; see Table 3.2).

Table 3.2. Statistics for each bivariate relationship in the structural equation models (SEM) for *Apis* and *Osmia*. The response and predictor variables are listed along with their correlation coefficients, standard errors, degrees of freedom, sample sizes, critical values, p-values, estimates, standardized correlation coefficients, significance levels, and transformations (response variables are listed first, followed by predictor variables). Significance symbology is as follows: 0.05<p<0.1=no symbol, 0.01<p<0.05=*, 0.001<p<0.01=**. Variables in italics represent independence claims, or direct effects. The "expectation" column delineates which relationships were predictions or alternatives (see Fig. 3.1).

SEM model	expectations	response	predictor	standard error	d.f.	n	critical value	p-value	estimate	standardized correlation coefficient	significance	transformation(s)
Apis	Prediction	proportion *Malus* pollen	apple land cover 3 km	33.624	11	13	-1.760	0.106	-59.167	-0.469		log+0.01, none
	Prediction	pesticide risk (%HQ)	proportion *Malus* pollen	1.110	11	13	-0.851	0.413	-0.944	-0.248		log+0.01, log+0.01
	Alternative	*pesticide risk (%HQ)*	*apple land cover 3 km*	*220.460*	*11*		*0.542*	*0.599*	*119.476*			*log+0.01, none*
Osmia	Prediction	proportion *Malus* pollen	apple land cover 1 km	8.476	12	14	2.221	0.046	18.821	0.540	*	log+0.01, none
	Prediction	pesticide risk (%HQ)	proportion *Malus* pollen	5.163	12	14	3.590	0.004	18.532	0.720	**	none, log+0.01
	Alternative	*pesticide risk (%HQ)*	*apple land cover 1 km*	*164.163*	*10*		*0.513*	*0.619*	*84.282*			*none, none*

0.008 se). The incidence of sites where bees collected zero *Malus* pollen did not differ between species, meaning our results were not influenced by null values (X^2=0, df = 1, p-value = 1).

In support of our prediction, we found that the mean pesticide concentration in *Osmia* pollen provisions was more than triple (781.353 ± 275.329, n=13) the concentration found in *Apis* beebread (217.850 ± 72.764 se, n=13), and the mean pesticide risk was over 1.85 times higher in *Osmia* pollen provisions (32.305 ± 12.305 se, n=13) than it was in *Apis* beebread (17.496 ± 9.993 se, n=13). However, there was no significant difference between the species for either pesticide exposure (V=55, p=0.112, n=13; Fig. 3.3B) or pesticide risk levels (t=0.970, df=12, p=0.176, n=13; Fig. 3.3D). The incidence of sites where pesticide exposure, and also risk, was not detected in bee-collected pollen did not differ between species (X^2= 0.99048, df = 1, p-value = 0.3196), suggesting that our results were not influenced by null values.

Discussion:

Our results indicate that *A. mellifera* is not an adequate predictor of *O. cornifrons* pesticide risk in apple agroecosystems. Even though there was no statistical difference in pesticide risk levels between species,, *Osmia* encounteredrisk above 25 %HQ (12.5% expected mortality in terms of honey bee topical LD_{50}) at six of the 14 sites, while average risk levels in *Apis* beebread only exceeded 25 %HQ at two of the 14 sites. Both species encountered risk at levels that could potentially impact their performance and, thus, pollination services. More importantly, the mechanisms through which the two species encountered pesticide risk were distinct. Pesticide risk levels in *Osmia* pollen provisions were driven by cropland cover and the proportion *Malus* pollen collected, while neither of these factors influenced pesticide risk to *Apis*. Our results suggest that the differences we found between species are influenced by differences in the *Malus* foraging radii and crop pollen collection between species, differences which likely reflect their distinct innate foraging ranges and pollen preferences.

As predicted, *Osmia* collected a greater proportion of crop (*Malus*) pollen than did *Apis.* A study comparing bee pollen loads corroborates our results, showed that *O. cornifrons* collected a greater higher mean proportion apple pollen (0.76) than *A. mellifera* (0.70) when foraging in apple, although this difference was not statistically significant (Russo, Park, Blitzer, & Danforth, 2017). Also mirroring our results, honey bee crop pollen collection near maize fields did not exceed 4% of beebread, even when proportion maize in the landscape was as high as 30%, likely due to honey bee preference for alternative pollen resources (Urbanowicz et al., 2019). Indeed, focal crop pollen collection by honey bees across seven different cropping systems was lower than 10% in beebread for five of the seven crops, although, contrary to what we found with *Malus* pollen, pollen collection for apple was around 75% (Pettis et al., 2013). The difference that we found in the proportion *Malus* pollen collected between species could be due to the marked preference that *Osmia* have for Rosaceous pollen types (Batra, 1978). However, it is also true that *Osmia* bees have been shown to collect only 14 plant genera in a season (Kraemer & Favi, 2005), compared with 36 collected by *Apis* (Synge, 1947). Thus, their narrower diet breadth and preference for Rosaceae pollen could have constrained *Osmia* bees to collect *Malus* (likely apple) crop pollen, especially if alternative resources were less ideal in comparison with nutritionally rich Rosaceous resources (Di Pasquale et al., 2013). It also seems likely that the difference in *Malus* foraging radii between the species influenced the proportion *Malus* pollen they collected. Indeed, within their respective foraging radii, the proportion apple land cover available to each species was as high as 18% for *Osmia*, but did not exceed 2% for *Apis*. A combination of floral resource preference, diet breadth, and foraging range could explain the differences we found in crop pollen collection between species.

Because our analysis was limited to contact LD_{50} values for *A. mellifera*, we cannot be certain that pesticide risk levels are accurate for oral larval exposure, especially for *Osmia*, as toxicity levels have been shown to vary between *O. cornifrons* and *A. mellifera*, depending on the pesticide (Biddinger et al., 2013). However, the levels of pesticide risk reported here are likely a conservative estimate of risk to

both species. For instance, our analysis assumes risk is additive when, in fact, synergism between pesticides can result in higher risk than expected (Sanchez-Bayo & Goka, 2014). Also, the %HQ in our study estimated risk based on mortality, but pesticides at field-realistic levels have been shown to have sublethal effects on bees, such as reduced pollen collection or nest recognition (Artz & Pitts-Singer, 2015; Gill et al., 2012). Finally, our study only tested for 11 active ingredients of the 47 that were reportedly sprayed by apple growers during bee activity, meaning we did not account for three-fourths of the potential pesticide exposure bees may have encountered. On the other hand, it should be noted that our selection of the lowest available contact LD_{50} might over-estimate risk.

The pesticide risk levels we found in bee-collected pollen likely have impacts on performance for both species. Indeed, across species, the %HQ in *Osmia* pollen exceeded the US Environmental Protection Agency acute contact level of concern at five of the 14 sites (US EPA, 2014). This threshold represents 20% expected mortality to honey bees exposed via acute contact (see %HQ equation in Methods). Though our risk calculation cannot directly predict mortality, negative effects on bee performance seem likely, as a previous study found weight reductions in *O. cornifrons* exposed to less than $1/100^{th}$ (%HQ=0.34) of the EPA threshold (Centrella et al., in prep). It is also possible that fungicides, which were found in substantial concentrations in bee-collected pollen despite their negligible contribution to %HQ, could have sublethal impacts to bees not accounted for in our assessment of "risk". In fact, pre-bloom fungicides have been shown to reduce wild bee richness and abundance in apple orchards (Park, Blitzer, Gibbs, Losey, & Danforth, 2015). Also, recent evidence shows that larval *O. ribofloris* exhibit reduced growth rate, biomass, and survivorship when deprived of pollen-borne microbes (Dharampal et al., 2019), suggesting that fungicide exposure could reduce bee fitness by sterilizing the microbial fauna in their pollen provisions.

Levels of pesticide risk and exposure in bee-collected pollen for *Osmia* and *Apis* were not significantly different. There was one site where both species encountered no pesticide exposure and,

thus, no pesticide risk in their pollen. This site was organic, but management does not seem to explain lack of pesticide exposure, because bees were exposed to pesticides in pollen at our 2 abandoned orchards, which receive no management. We also expected that, because of their larger foraging range and broader diet breadth, pesticide exposure from apple sprays would be reduced for *Apis*. However, the proportion pesticide risk coming from outside of the orchards did not differ between species, and over 80% of pesticide risk in *Apis* beebread came from active ingredients reportedly sprayed *inside* of the focal apple orchards. These unexpected results can be explained by the possibility that apple orchard sprays contaminated other flowers in the apple understory or adjacent to the orchards. In fact, drift from the focal crop can occur up to 1000 m (Woods et al., 2001), and neonicotinoid residues from crop treatments have been found in nearby dandelions and in the pollen and nectar of wildflowers along crop margins (Botias et al., 2015; Krupke, Hunt, Eitzer, Andino, & Given, 2012). It is also possible that the active ingredients sprayed by apple growers are not apple specific and were also sprayed in other crops within the foraging radius of *Apis*. Indeed, of the 11 active ingredients detected in bee pollen that were also reportedly sprayed in apple, at least five of them are broad spectrum and registered in a variety of crops in New York State (NYSPAD, 2019). Thus, it is possible that honey bees are avoiding pesticide risk from apple sprays, but encountering similar levels of the same active ingredients used in other spring-blooming crops within their foraging radius. Although Brittain & Potts (2011) suggested that bees with smaller foraging ranges encounter higher pesticide risk because they cannot escape the dominant crop near where they are nesting, it also seems plausible that a larger foraging range may lead to at least equal exposure to pesticides used across multiple cropping systems.

As predicted, the proportion apple land cover surrounding our sites indirectly correlated with increased pesticide risk in *Osmia* pollen provisions, via increased proportion *Malus* pollen. Previous studies have shown that increasing agriculture in the landscape can negatively impact wild bee body size and visitation rates (Nicholson, Koh, Richardson, Beauchemin, & Ricketts, 2017; Renauld, Hutchinson,

Loeb, Poveda, & Connelly, 2016). Our study suggests that one of the mechanisms explaining these patterns could be increased pesticide risk through crop pollen collection. However, further study corroborating our results is necessary, as our analysis with apple land cover was driven by an outlier (see Results). Contrary to our prediction, for *Apis*, the proportion apple land cover within their *Malus* foraging radius did not appear to significantly influence pesticide risk levels in their beebread: neither directly, nor through *Malus* pollen collection. This result is not unprecedented. A recent study of pesticide risk to honey bees in maize showed that the proportion of maize in the landscape did not predict maize pollen collection or pesticide risk in *A. mellifera* beebread (Urbanowicz et al., 2019). In addition, honey bees can encounter high levels of pesticide exposure and risk in their beebread even when focal crop collection is low (Long & Krupke, 2016; McArt et al., 2017), suggesting that focal crops are not necessarily major drivers of honey bee pesticide risk.

Though unexpected, our finding that apple land cover is associated with pesticide risk for *Osmia* but not for *Apis* can be explained by differences in *Malus* pollen collection and *Malus* foraging radius between species. As apple land cover available to *Osmia* increased, they collected a higher proportion *Malus* in their pollen, but *Apis* showed no such relationship, suggesting that innate Rosaceae preference could be driving pesticide risk to bees. However, it is also true that, at their larger *Malus* foraging radius, *Apis* encountered a much lower maximum proportion of apple in the landscape than did *Osmia* (see above), meaning it is possible that, in landscapes more dominated by apple cropland, we might observe a similar relationship with *Apis* risk as we did with *Osmia*.

Malus pollen collection and *Malus* foraging radius are likely driven by innate pollen preferences and foraging ranges in both species. However, the link between these traits and our results is not studied here, nor are we able to disentangle the effects of crop pollen collection and *Malus* foraging radius from one another. It seems likely, however, that both traits are influencing our results. Similar to our study, a meta-analysis across five studies found that increasing proportion crop area in the

landscape had a negative effect on solitary bee abundance, but had no effect on social bee abundance; similar patterns were found for bee diversity (Coutinho, Garibaldi, & Viana, 2018). This suggests that wild bees may be more sensitive to the proportion cropland than honey bees, and our study is evidence that this could be driven by differences in crop pollen collection, foraging range, and pollen preferences between wild bees and honey bees. Our study suggests that innate differences in foraging range and pollen preference may render solitary, wild bees more sensitive to pesticide exposure and risk than honey bees. This has important implications for how we assess pesticide risk to bees in agroecosystems.

Implications:

Our results indicate that *A. mellifera* is not an adequate predictor of wild bee pesticide risk, at least for *O. cornifrons* in apple agroecosystems, because honey bees were less influenced by cropland cover and crop pollen collection than wild *Osmia* bees. We show that wild bee sensitivity to agricultural cropland was associated with (1) a smaller crop foraging radius, likely reflective of innate foraging range, and (2) increased crop pollen collection, likely reflective of diet preference, compared to honey bees. Many wild bees have significantly smaller foraging ranges than honey bees. Ten common European solitary bees have foraging ranges smaller than 300 m (Gathmann & Tscharntke, 2002). When these bees are nesting in agroecosystems, we might expect them to experience very high levels of pesticide exposure and risk simply because their foraging range is restricted. Many wild bees also have an extremely narrow range of host-plant preferences. Indeed, we would expect oligolectic, specialist bees to encounter extremely high pesticide exposure if their host-plants are also crops. For example, pollen loads from *Andrena miserabilis*, a common wild bee in eastern apple orchards, revealed that 80% of their pollen load collected in apple was apple pollen (Russo & Danforth, 2017), likely leading to high levels of pesticide exposure. Likewise, the squash bee (*Peponapis pruinosa*) forages exclusively on squash pollen (López-Uribe et al., 2016) and are likely encountering high levels of agrochemicals applied to squash fields in their pollen. According to our results, the combination of a small foraging range and

narrow host-plant preference could have significant impacts on certain bee species in agroecosystems. To preserve pollination services in agroecosystems where we most rely on wild bee pollinators, it is imperative that we study the ways in which *multiple* bee species across a variety of cropping systems encounter pesticide risk, because *A. mellifera* is not a reliable model for *how* all bees encounter pesticide risk.

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APPENDIX 1: SUPPLEMENTARY MATERIALS FOR CHAPTER 1

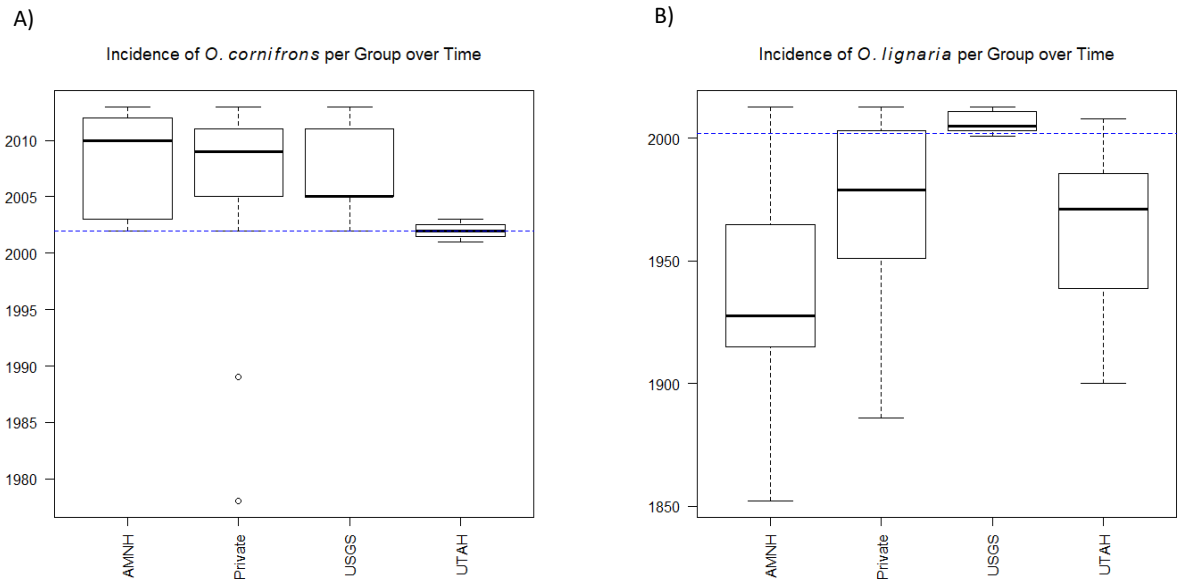

Figure A1.1. Analysis of data per collection group for *O. lignaria* (A) and *O. cornifrons* (B), including the top three repositories (or 18 collections, 16 from AMNH) and the combination of the remaining 18 private collections. For the number of UCEs per collection, see Table A1.2. Dashed blue lines represent *O. cornifrons* detection in the collections (2002).

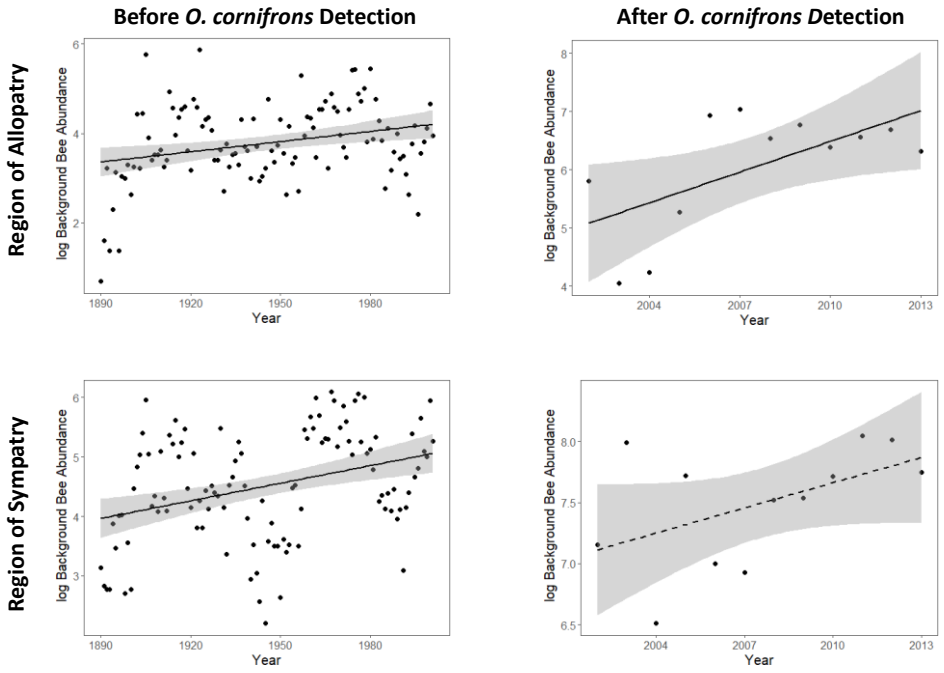

Figure A1.2. Background bee UCE abundance over time in the regions of allopatry and sympatry, both before and after *O. cornifrons* detection. Over time, the background bee abundance increased significantly in the region of allopatry before detection (R2=0.061, F1,110=8.265, p=0.005), in the region of allopatry after detection (R2=0.3243, F1,10=6.279, p=0.031), and in the region of sympatry before detection (R2=0.111, F1,110=14.87, p<0.001), while it increased only marginally significantly in the sympatric region after *O. cornifrons* detection (R2=0.181, F1,10=3.435, p=,0.095), Points represent the yearly summed background bee UCEs, smooth lines show significant relationships and dashed lines marginal relationships, and grey shading about lines shows the 95% confidence interval.

Table A1.1. UCE sample size for *O. lignaria, O. cornifrons* and background bees in the regions of allopatry and sympatry, both before and after *O. cornifrons* detection in collections (2002). Original specimen count is shown in "specimens" column. "18C" denotes the subset of data from the 18 collections used for relative abundance analysis. All other data is based on the 36 collections. Here, "Background Bees" represent the combination of all bees *plus* all *O. lignaria* and *O. cornifrons* (see Statistics section).

Group	***Specimens***	***UCEs***	***1890-2013***	***Sympatry Before***	***Sympatry After***	***Allopatry Before***	***Allopatry After***
O. lignaria 36C	*2921*	*1744*	*1732*	*434*	*227*	*395*	*48*
O. lignaria 18C	*1324*	*1016*	*1014*	*412*	*208*	*172*	*21*
O. cornifrons 36C	*721*	*407*	*407*	*3*	*399*	*0*	*0*
O. cornifrons 18C	*627*	*367*	*367*	*1*	*361*	*0*	*0*
Background Bees	*260747*	*86750*	*85843*	*14804*	*23763*	*7197*	*7053*

Table A1.2. The 36 collections from which *O. cornifrons* and *O. lignaria* specimens were sourced for our study, including the collection location, parent institution, parent repository (if applicable), specimen format, and the number of specimens (after data set refining) for each species. For collections that were already part of the AMNH repository, the "Additional Data Sought" column shows whether new physical specimens were catalogued as part of this project. For some collections that were already part of the AMNH repository, additional digital specimens were sought to achieve more extensive, current records. These additional difital specimens were not uploaded to AMNH for this project. All physical specimens were added to the AMNH repository as part of this project. The collection codes listed here were used as identifiers throughout the study and correspond to Fig. A1.1.

Code	*Collection*	*Locality*	*Parent Institution*	*Parent Repository*	*Specimen Format*	*Additional Data Sought*	*# O. cornifrons*	*# O. lignaria*
AMNH	*American Museum of Natural History*	*New York, NY*		*AMNH*	*Digital*		*86*	*201*
BRFC	*Black Rock Forest Collection*	*Cornwall, NY*		*AMNH*	*Digital*		*8*	*0*
CAES	*Connecticut Agricultural Experiment Station*	*New Haven, CT*		*AMNH*	*Digital*		*18*	*6*
CUIC	*Cornell University Insect Collection*	*Ithaca, NY*		*AMNH*	*Digital*		*41*	*107*
HZIC	*Harry Zirlin Insect Collection*	*NJ and NY*		*AMNH*	*Digital*		*7*	*0*
JSAOBS	*John S. Ascher Bee Observation Database*	*New York, NY*		*AMNH*	*Digital*		*1*	*3*
MMWVU	*Mathew McKinney's Private Collection*	*Morgantown, WV*	*West Virginia University*	*AMNH*	*Digital*		*0*	*1*
NCSU	*North Carolina State University*	*Raleigh, NC*		*AMNH*	*Digital*	*Physical*	*2*	*114*
NDBC	*Nelson DeBarros' Private Collection*	*CT & MA*		*AMNH*	*Digital*		*1*	*0*
NYSM	*New York State Museum Entomological Collection*	*Albany, NY*		*AMNH*	*Digital*		*0*	*23*
OSU	*C.A. Triplehorn Insect Collection*	*Columbus, OH*	*The Ohio State University*	*AMNH*	*Digital*	*Digital*	*0*	*14*
PSUC	*Frost Entomological Museum*	*State College, PA*	*Pennsylvania State University*	*AMNH*	*Digital*	*Physical*	*21*	*22*
RMBL	*Rocky Mountain Biological Lab*	*Crested Butte, CO*		*AMNH*	*Digital*		*0*	*4*
RUAC	*Rutgers University*	*Piscataway Township, NJ*		*AMNH*	*Digital*		*0*	*46*
SIIS	*Staten Island Institute of Arts and Sciences*	*Staten Island, NY*		*AMNH*	*Digital*		*0*	*2*
UCMS	*University of Connecticut Museum at Storrs*	*Storrs, CT*		*AMNH*	*Digital*		*6*	*25*
UDCC	*University of Delaware*	*Newark, DE*		*AMNH*	*Digital*	*Physical*	*37*	*1*
UNHP	*University of New Hampshire Insect Collection*	*Durham, NH*		*AMNH*	*Digital*		*0*	*19*

Table A1.2 (Continued).

Code	*Collection*	*Locality*	*Parent Institution*	*Parent Repository*	*Specimen Format*	*Additional Data Sought*	*# O. cornifrons*	*# O. lignaria*
USNM	*United States National Museum*	*Washington, D.C.*		*AMNH*	*Digital*	*Physical*	*4*	*271*
FMNH	*Field Museum of Chicago*	*Chicago, IL*			*Physical*		*2*	*14*
HMCZ	*Harvard Museum of Comparative Zoology*	*Cambridge, MA*			*Physical*	*Digital*	*0*	*18*
ILL	*Illinois Natural History Survey*	*Champaign, IL*			*Digital*		*0*	*205*
INDR	*Robert Jean's personal collection*	*Saint Mary-of-the-Woods, IN*	*Saint Mary-of-the-Woods College*		*Digital*		*0*	*127*
JGIBBS	*Jason Gibbs' private collection*	Winnepeg, MB			*Digital*		*0*	*13*
JMILAM	*Joan Milam's personal collection*	*Amherst, MA*			*Digital*		*18*	*1*
MFVEIT	*Michael Veit's private collection*	*Pepperell, MA*			*Digital*		*13*	*7*
MICHGIB	*AJ Cook Arthropod Collection*	*East Lansing, Michigan*	*Michigan State University*		*Digital*		*1*	*96*
MICHST	*University of Michigan Museum of Zoology*	*Ann Arbor, Michigan*			*Digital*		*12*	*84*
NAAD	*Nancy Adamson's private collection*	*Greensboro, NC*			*Digital*		*47*	*33*
ROM	*Royal Ontario Museum*	*Toronto, ON*			*Digital*		*0*	*16*
SOPCAR	*Canada National Collection*	*Ottawa, ON*			*Digital*		*0*	*596*
UCANR	*Miriam Richards' private collection*	*ON, multiple locations*			*Digital*		*0*	*8*
UGUEL	*University of Guelph Insect Collection*	*Guelph, ON*			*Digital*		*0*	*16*
UKSJ	*University of Kansas*	*Lawrence, KS*			*Digital*		*3*	*363*
USGS	*Sam Droege's Collection*	*Patuxent Wildlife Preserve, MD*	*United States Geological Survey*		*Digital*		*393*	*197*
UTAH	*United States Department of Agriculture Collection*	*Logan, UT*			*Digital*		*0*	*268*

Table A1.3. Comparison of precipitation ("Precip") and temperature in the region of allopatry and the region of sympatry. Summed precipitation (mm) and mean temperature (˚C) for the month of April were averaged per year. Means across years are presented here plus or minus their standard errors, and the difference between regions is shown as the mean for the region of sympatry subtracted from the mean from the region of allopatry. Precipitation and temperature meansaveraged across 6 three-year periods from 2001 to 2013 and across 11 ten-year periods from1885 to 1995 are shown in the upper and lower panels, respectively.

		Precip	**Temp**
		mm	C
2001-2013 *n=6*	**Sympatry**	106.0 (±17.64)	10.7 (±0.27)
	Allopatry	110.0 (±17.40)	11.0 (±0.41)
	allopatry-sympatry	*4*	*0.3*
1885-1995 *n=11*	**Sympatry**	71.5 (±7.20)	10.2 (±0.71)
	Allopatry	77.8 (±8.11)	11.0 (±0.66)
	allopatry-sympatry	*6.3*	*0.8*

Table A1.4. Vertices for the polygons created east of -90 DD that encompassed all *O. cornifrons* specimens (left) and *O. lignaria* specimens (right). The region of allopatry was the portion of the *O. lignaria* polygon that did not overlap with the *O. cornifrons* polygon, or the region of sympatry (see Fig. 1.2 for graphical representation).

***O. cornifrons* polygon**			***O. lignaria* polygon**	
X	*Y*		*X*	*Y*
-76.822400	*38.407900*		*-86.81300*	*33.52500*
-72.179850	*41.490700*		*-84.38805*	*33.74888*
-70.850670	*43.083050*		*-76.74023*	*34.69628*
-70.847500	*43.093200*		*-66.64396*	*45.96340*
-83.715060	*42.256900*		*-88.86667*	*49.36667*
-83.984600	*35.508700*		*-89.33070*	*46.28650*
-82.501230	*35.430690*		*-89.88167*	*39.27972*
			-89.85000	*35.36667*
			-88.81830	*33.45030*

APPENDIX 2: SUPPLEMENTARY MATERIALS FOR CHAPTER 2

Appendix A2.1. Materials and methods concerning additional bee response variables and 2014 floral surveys.

Proportion larval mortality (of offspring) was measured as the number of unconsumed pollen provisions and/or larval bees divided by the total *Osmia* cells produced, determined with x-ray images taken once bees had undergone metamorphosis (Dec 16, 2015). The proportion of female offspring (or sex ratio) produced was measured as the number of females emerged divided by the total bees emerged. For male offspring weight [Mettler Toledo MS105DU Semi-Micro Balance], two time points were accidentally combined, and at a third time point, no males emerged, so analysis was based on 48 observations. For proportion larval mortality, only 50 observations were analyzed, due to removal of an outlier confirmed to be statistically significant using Grubbs' single outlier test (Tmax= 4.793303, $p<0.025$, df=50). Variable selection and path model tests were conducted for each response variable following the procedures in the *Statistics* section of the Methods section of Chapter 2. Family evenness was negatively skewed and thus squared. The insecticide risk data had strong positive kurtosis, which was reduced with a log plus one transformation. Fungicide risk had both positive kurtosis and was positively skewed, properties which were not reduced by a log transformation and could only be rectified with a sixth-root transformation. The number of males was measured as integer (count) data. However, Poisson and quassi-Poisson models for this variable were over-dispersed, so it was square-root transformed. Proportion females was arc-sine transformed, a common transformation for proportional data. Proportion larval mortality data had positive kurtosis and was square-root transformed. Due to positive skew, male weight was cube-root transformed.

Initial path models based on our hypothesized relationships (Fig. 2.1) were consistent with the data (number of male offspring: Fischer's C=10.811, p=0.545, df=12; proportion female offspring: Fischer's C=3.15, p=0.994, df=12; proportion larval mortality: Fischer's C=4.206, p=0.979, df=12; male offspring weight: Fischer's C=4.186, p=0.98, df=12), but model fit, in terms of AIC-value, was improved (see delta AIC values in Fig. A2.1 caption) by iterative removal of non-significant (p>0.1) relationships. Proportion larval mortality and male weight had just sufficient sample size for the path analysis, but were close to the cut-off value (see the Methods section of Chapter 2 for further explanation of sample size and piecewise SEM theory). For this reason, Monte Carlo simulations were conducted, resulting in chi-square probabilities of 0.984 for the initial path model of proportion larval mortality, and 0.983 for the initial male weight path model. These results provide strong evidence that the path analysis results we present here were achieved with sufficient power.

Weekly floral surveys were conducted from April through October, 2014 at 15 of our 17 sites. Surveys consisted of three, 50 m transects at each site, one along the orchard perimeter and two within the orchard. Three-dimensional floral abundance attributed to each flowering plant was estimated in 10 m segments along transects. Floral richness was assessed in an exhaustive, species-level survey across the entire orchard and its perimeter. Specimen vouchers were collected, plants were identified, and associated pollen grains were stained and mounted on slides following procedures in Chapter 2. Floral survey data were compared to 2015 data for the 15 overlapping sites using linear mixed models, statistics, and normality criteria described in Chapter 2. In all models, site was included as a random effect. For the model in which floral abundance was a response, floral abundance was natural log transformed.

Figure A2.1. Final path models for additional bee response variables. These include the number of males (A; final model: Fisher's C=11.869, p=0.617, df=14, n=51, Δ AIC from initial model=-8.942), proportion female offspring (B; Fisher's C=6.417, p=0.99, df=18, n=51, Δ AIC from initial model=-8.733), proportion larval mortality (C; Fisher's C=4.874, p=0.996, df=16, n=50, Δ AIC from initial model=-5.332), and male weight (D; Fisher's C=2.155, p=0.905, df=6, n=48, Δ AIC from initial model=-34.031). Unidirectional arrows represent supported relationships (red negative, black positive) between variables (in boxes). Links found in a priori model may be omitted here because their removal increased model fit. Arrows are scaled to the magnitude of the standardized correlation coefficients, shown in boxes alongside arrows along with p-value significance levels (0.05<p<0.1=no symbol, 0.01<p<0.05=*, 0.001<p<0.01=**, p<0.001=***). Semi-transparent arrows represent non-significant (p>0.1) relationships that still supported the model fit (based on AIC score). For clarity, the variables "Number of Pollen Provisions" (# Provisions), "Temperature", and "Time Point" have been omitted and instead their correlation coefficients are shown in italics next to boxes of associated response variables (magnitude not shown for non-significant relationships). Numbers next to landscape categories represent the scale, or radius in meters about sites. Statistics are based on transformed variables (see Table A2.1).

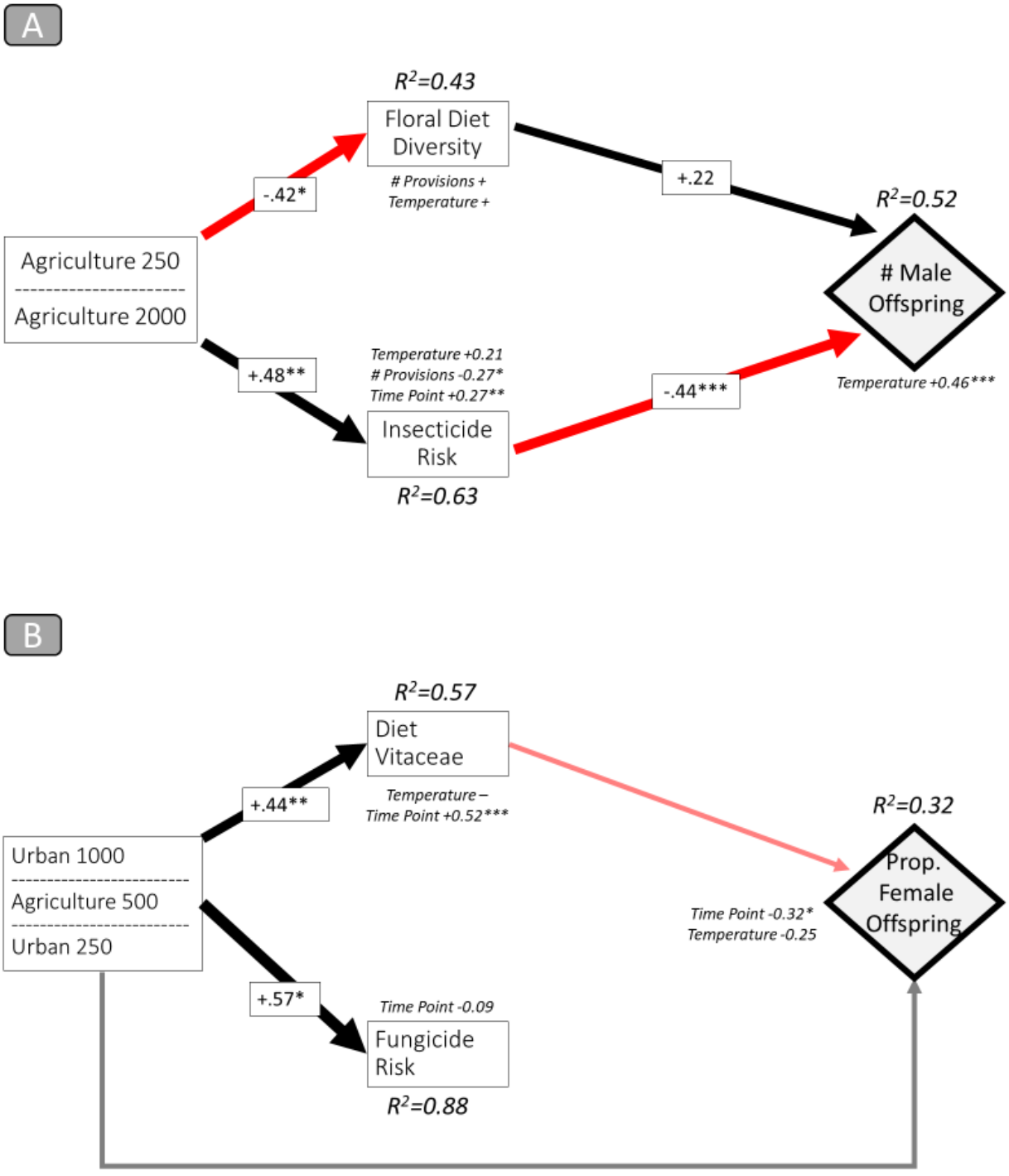
A
R^2=0.43
Floral Diet Diversity
Provisions +
Temperature +
-.42*
+.22
R^2=0.52
Male Offspring
Agriculture 250
Agriculture 2000
+.48**
Temperature +0.21
Provisions -0.27*
Time Point +0.27**
-.44***
Temperature +0.46***
Insecticide Risk
R^2=0.63
B
R^2=0.57
Diet Vitaceae
Temperature –
Time Point +0.52***
+.44**
R^2=0.32
Prop. Female Offspring
Urban 1000
Agriculture 500
Urban 250
Time Point -0.32*
Temperature -0.25
+.57*
Time Point -0.09
Fungicide Risk
R^2=0.88

Figure A2.1 (Continued).

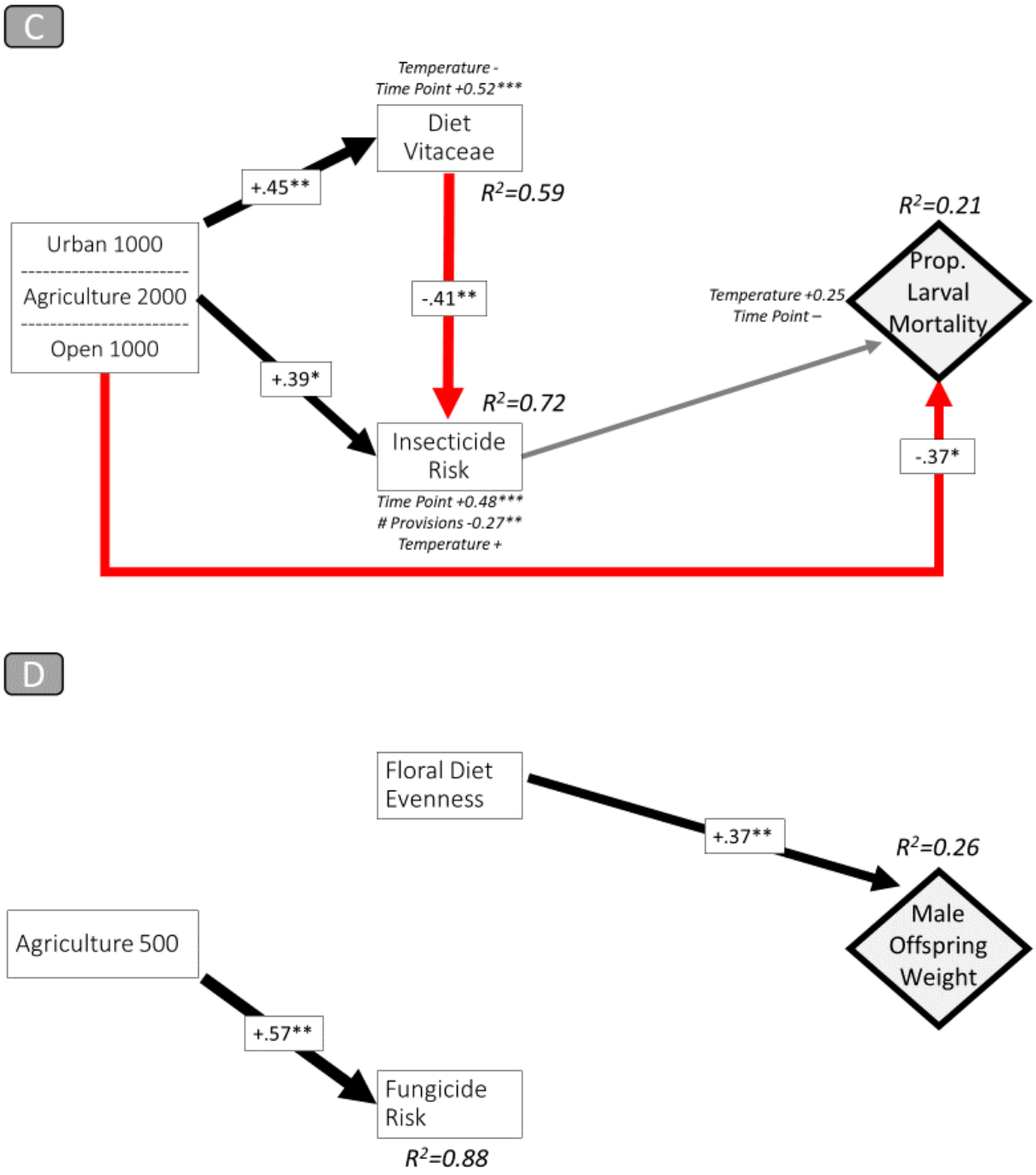

Figure A2.2. Mason bee nesting materials. A mason bee nesting shelter (top left). Completed nest tubes (bottom left) were collected for offspring and pollen analysis. A nest tube dissection (right) reveals pollen provisions and attached eggs. Larger provisions towards the back of the nest indicate female offspring, while smaller provisions in front are likely males.

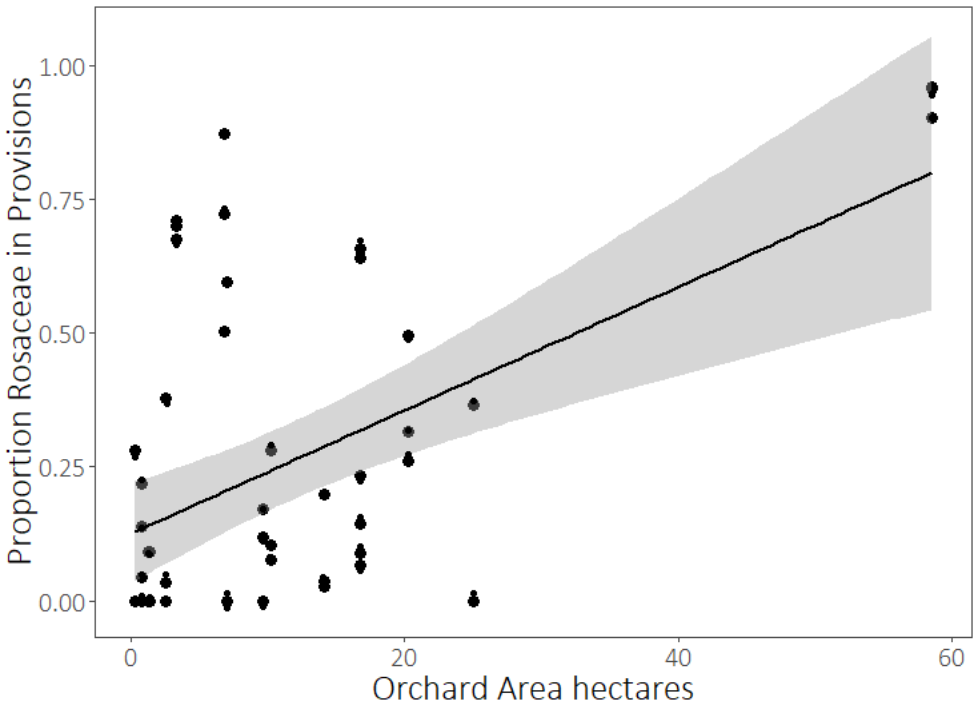

Figure A2.3. The relationship between Rosaceae collected in pollen provisions and orchard size. The linear relationship between the proportion Rosaceae in bee-collected pollen per time point and orchard area in hectares. The variables site and time point were included as random and fixed effects. As orchard area increased, the proportion Rosaceae collected in the pollen provisions also increased ($F_{1,15}$=7.88, p=0.013, n=51), suggesting that Rosaceae pollen is at least in part apple pollen. Points represent time-points, with size and position (<0.001 proportion) altered for visual transparency. The shadow about the line represents the 95% confidence interval.

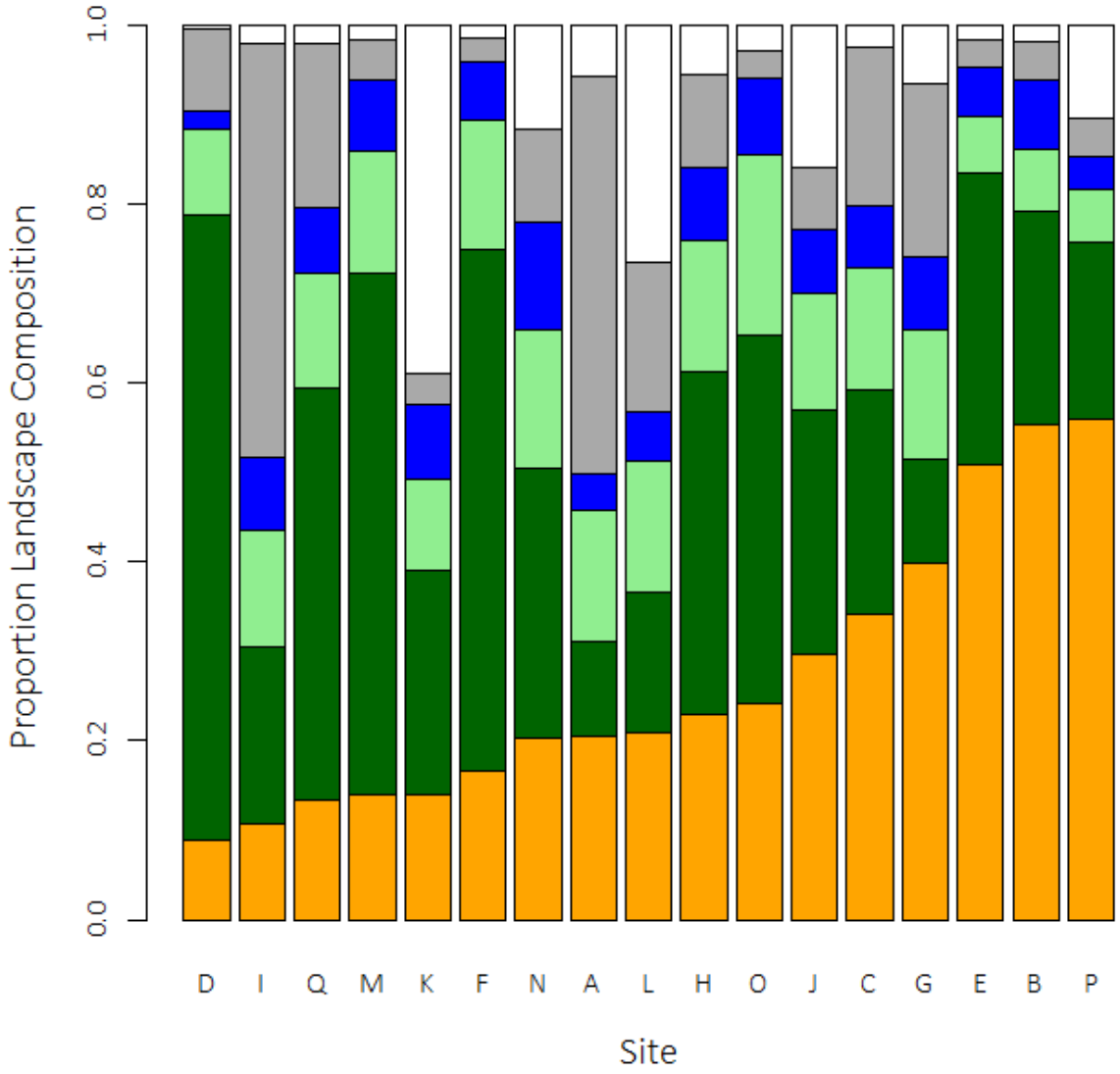

Figure A2.4. Summary of landscape composition per site. Landscape composition surrounding our 17 sites (letters) at a 2 km radius. Proportion surrounding agriculture (orange), forests (dark green), open areas (light green), shrub/wetlands (blue), urban areas (dark grey), and other (white) are presented here in ascending order of proportion agriculture.

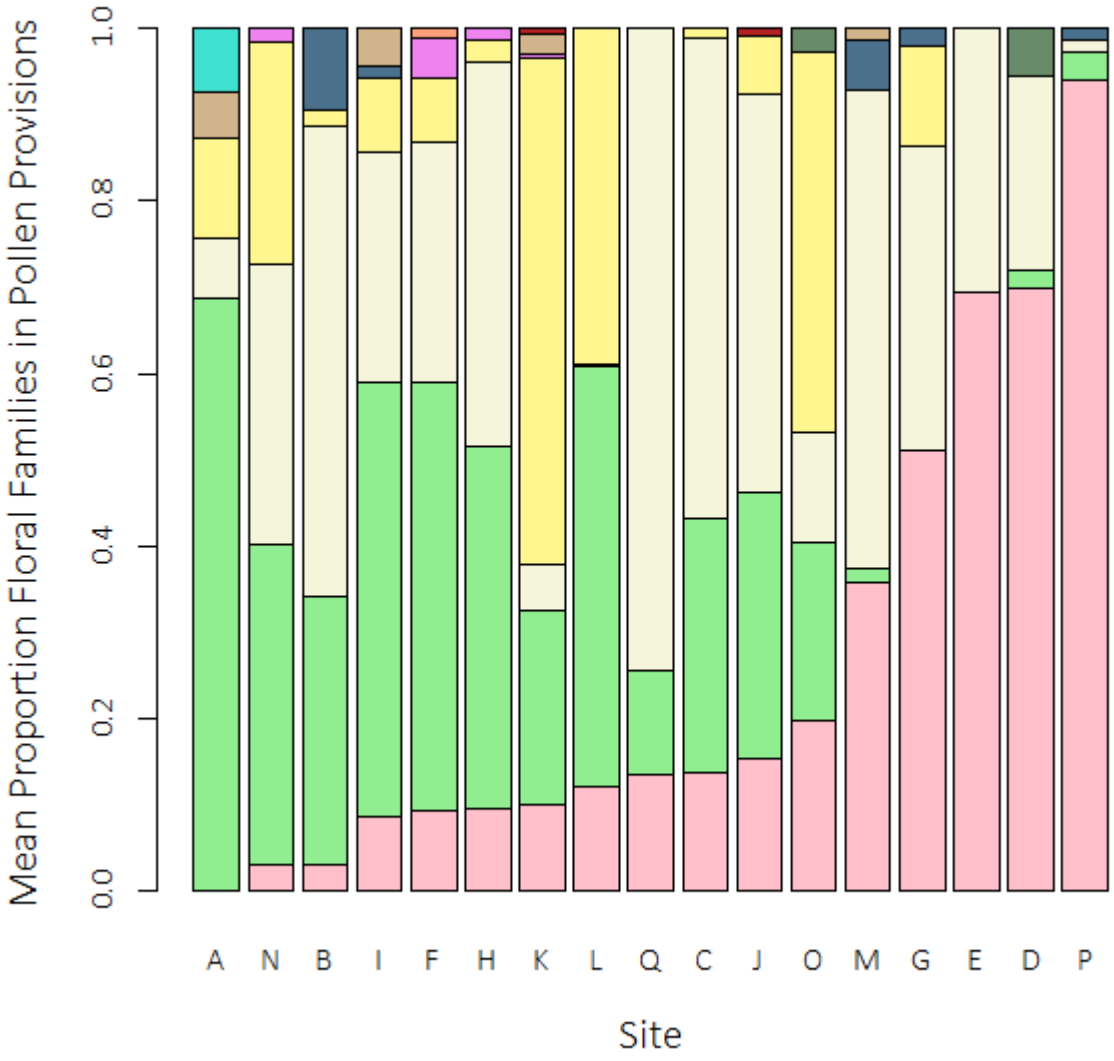

Figure A2.5. Summary of diet diversity in provisions. The proportion of floral families collected in bee pollen averaged per site (letters), in increasing order of proportion Rosaceae (pink). There were 11 total floral families collected across our 17 sites: Rosaceae (pink), Vitaceae (light green), Caprifoliaceae (beige), Rhamnaceae (yellow), Cornaceae (slate blue), Onagraceae (purple), Juglandaceae (brown), Oleaceae (turquoise), Fabaceae (orange), Adoxaceae (dark green), and Brassicaceae (dark red). Only 4 families were found at 10 or more sites: Vitaceae in light green (found at 30 time points, ranging from 0.043 to 0.838, mean where found: 0.451 ± 0.051 se), Caprifoliaceae in beige (found at 44 time points, ranging from 0.007 to 0.955, mean where found: 0.363 ± 0.043 se), Rhamnaceae in yellow (found at 24 time points, ranging from 0.017 to 0.862, mean where found: 0.273 ± 0.057 se), and Rosaceae in pink (found at 36 time points, ranging from 0.026 to 0.96, mean where found: 0.365 ± 0.050 se). Floral family richness ranged from 2 to 7 families collected per time point (mean 3.14 ± 0.161 se). Floral Shannon diversity at the family level ranged from 0.169 to 1.343 per time point (mean 0.741 ± 0.040 se). Floral family evenness ranged from 0.24 to 0.995 per time point (mean 0.687 ± 0.027 se).

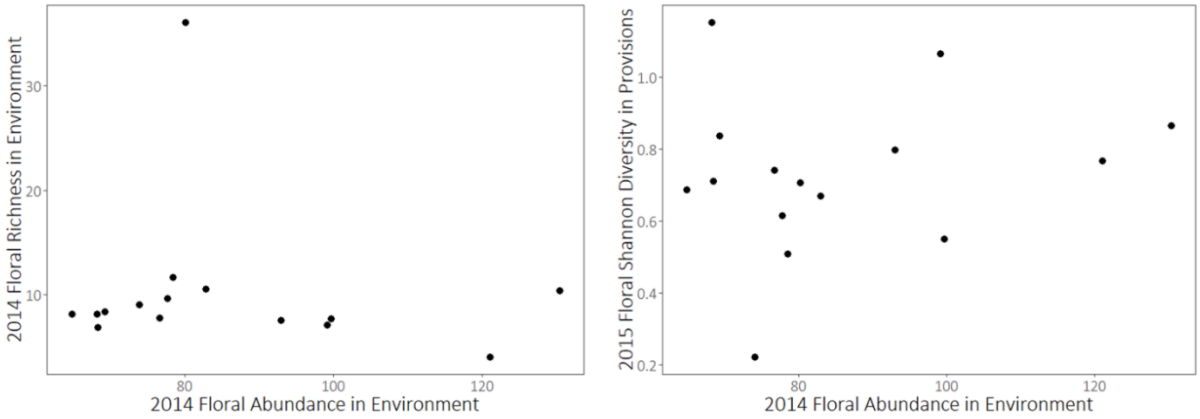

Figure A2.6. 2014 environmental floral resource survey results. A comparison of 2015 floral diet diversity and 2014 floral survey information from 15 of our sites. Points represent site averages. 2014 seasonal floral species richness in the environment varied between 62 and 361 flowering species per site. There is a non-significant relationship ($F_{1,13}$=0.210, p=0.655, n=15) between floral species richness and floral resource abundance in the environment (left panel) in 2014. Also, 2014 floral resource abundance in the environment does not predict floral Shannon diversity at the family level in pollen provisions in 2015 ($F_{1,13}$=0.415, p=0.531, n=15; middle panel). Plots show single predictor models, while statistics are based on models with the variable site as a random effect.

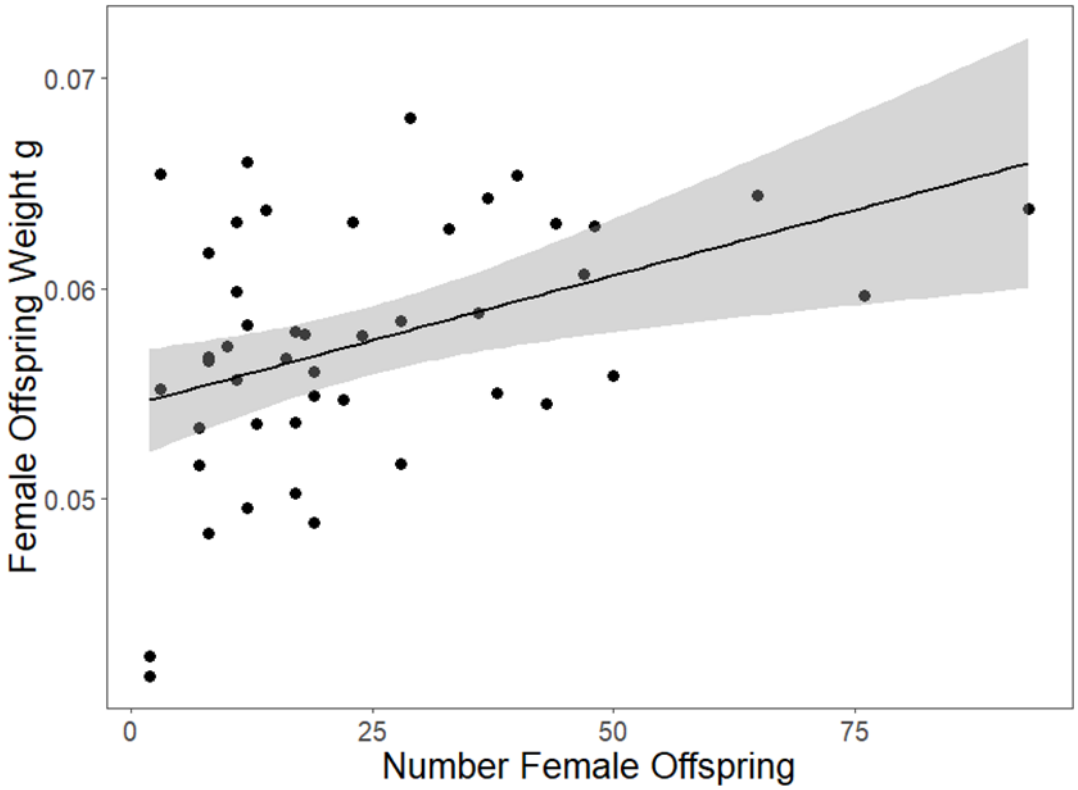

Figure A2.7. Relationship between bee response variables. There was a signifcant positive relationship between the number of female offspring produced at each time point and the weight of those offspring in grams ($F_{1,28}$=10.616, p=0.003, n=46). Points represent time point observations and the line represents a significant relationship. The grey shadow surrounding the line represents the 95% confidence interval. The variables site and time point were included as random and fixed effects in the analysis.

Table A2.1. Final structural equation model statistics for additional bee response variables. Statistics for final structural equation models for additional bee response variables, including number of male offspring, male offspring weight, proportion females, and proportion larval mortality. The response and predictor variables for each bivariate relationship supported by the SEM paths are listed, along with their correlation coefficients, corresponding standard errors (se), degrees of freedom (df), sample sizes (n), critical values, p-values, significance levels, and transformations (listed for response variables first, then predictor variables). Significance symbology is as follows: 0.05<p<0.1=no symbol, 0.01<p<0.05=*, 0.001<p<0.01=**, p<0.001=***. Variables in italics represent correlated errors.

SEM Model	Response	Predictor	se	df	n	Critical Value	p-value	Correlation Coefficient	Significance	Transformation(s)
Number Male Offspring	Number Male Offspring	Floral Shannon Diversity	0.784	31	48	1.953	0.060	0.218		square-root
	Number Male Offspring	Insecticide Risk (%HQ)	0.106	31	48	-4.060	0.000	-0.438	***	square-root, log+1
	Number Male Offspring	Temperature	0.057	31	48	4.333	0.000	0.462	***	square-root
	Floral Shannon Diversity	Agriculture 250 m	0.166	15	48	-2.680	0.017	-0.417	*	
	Floral Shannon Diversity	Temperature	0.010	32	48	0.982	0.333	0.128		
	Floral Shannon Diversity	Number Pollen Provisions	0.005	32	48	1.196	0.240	0.156		
	Insecticide Risk (%HQ)	Agriculture 2000 m	1.999	15	48	3.203	0.006	0.480	**	log+1
	Insecticide Risk (%HQ)	Time Point	0.224	31	48	2.904	0.007	0.266	**	log+1
	Insecticide Risk (%HQ)	Temperature	0.059	31	48	1.867	0.071	0.206		log+1
	Insecticide Risk (%HQ)	Number Pollen Provisions	0.032	31	48	-2.534	0.017	-0.274	*	log+1
	Number Male Offspring	*Number Pollen Provisions*	*NA*	*51*	*48*	*2.139*	*0.019*	*0.295*	*	*square-root*

Table A2.1 (Continued).

SEM Model	Response	Predictor	se	df	n	Critical Value	p-value	Correlation Coefficient	Significance	Transformation(s)
Proportion Female Offspring	Proportion Female Offspring	Temperature	0.007	31	51	-1.995	0.055	-0.248		arc-sine
	Proportion Female Offspring	Urban 250 m	0.262	15	51	1.693	0.111	0.209		arc-sine
	Proportion Female Offspring	Proportion Vitaceae	0.095	31	51	-1.587	0.123	-0.225		arc-sine
	Proportion Female Offspring	Time Point	0.036	31	51	-2.214	0.034	-0.316	*	arc-sine
	Proportion Vitaceae	Urban 1000 m	0.279	15	51	3.286	0.005	0.445	**	
	Proportion Vitaceae	Temperature	0.008	32	51	-1.270	0.213	-0.129		
	Proportion Vitaceae	Time Point	0.036	32	51	5.393	0.000	0.523	***	
	Fungicide Risk (%HQ)	Agriculture 500 m	0.221	15	51	2.931	0.010	0.575	*	6th-root
	Fungicide Risk (%HQ)	Time Point	0.016	33	51	-1.850	0.073	-0.093		6th-root
	Proportion Female Offspring	*Number Pollen Provisions*	*NA*	*51*	*51*	*0.953*	*0.173*	*0.136*		*arc-sine*

Table A2.1 (Continued).

SEM Model	Response	Predictor	se	df	n	Critical Value	p-value	Correlation Coefficient	Significance	Transformation(s)
Proportion Larval Mortality	Proportion Larval Mortality	Open 1000 m	0.411	15	50	-2.756	0.015	-0.369	*	square-root
	Proportion Larval Mortality	Insecticide Risk (%HQ)	0.013	30	50	0.970	0.340	0.137		square-root, log+1
	Proportion Larval Mortality	Temperature	0.007	30	50	1.817	0.079	0.246		square-root
	Proportion Larval Mortality	Time Point	0.031	30	50	-1.573	0.126	-0.224		square-root
	Insecticide Risk (%HQ)	Agriculture 2000 m	1.914	15	50	2.738	0.015	0.395	*	log+1
	Insecticide Risk (%HQ)	Proportion Vitaceae	0.789	29	50	-3.350	0.002	-0.406	**	log+1
	Insecticide Risk (%HQ)	Temperature	0.054	29	50	1.435	0.162	0.142		log+1
	Insecticide Risk (%HQ)	Time Point	0.248	29	50	4.741	0.000	0.483	***	log+1
	Insecticide Risk (%HQ)	Number Pollen Provisions	0.029	29	50	-2.801	0.009	-0.271	**	log+1
	Proportion Vitaceae	Urban 1000 m	0.288	15	50	3.222	0.006	0.449	**	
	Proportion Vitaceae	Temperature	0.008	31	50	-1.090	0.284	-0.110		
	Proportion Vitaceae	Time Point	0.036	31	50	5.407	0.000	0.520	***	
	Proportion Larval Mortality	*Number Pollen Provisions*	*NA*	*50*	*50*	*-0.153*	*0.439*	*-0.022*		*square-root*

Table A2.1 (Continued).

SEM Model	Response	Predictor	se	df	n	Critical Value	p-value	Correlation Coefficient	Significance	Transformation(s)
Male offspring Weight	Male Offspring Weight	Floral Evenness	0.007	30	48	2.781	0.009	0.369	**	cubed-root, squared
	Fungicide Risk (%HQ)	Agriculture 500 m	0.232	15	48	2.990	0.009	0.573	**	6th-root
	Male Offspring Weight	*Number Pollen Provisions*	*NA*	*48*	*48*	*-0.259*	*0.398*	*-0.039*		*cubed-root*

Table A2.2. Pesticides tested that were not detected above the limit of quantification**.** Here, we list,in alphabetical order, the 154 (of 188) active ingredients that were tested but were *not* found above their average limit of quanitification (LOQ) across three sets of *Osmia* pollen provision samples.Quantification limits are shown here (in ppb) and estimates are based on pesticides spiked into 5 g honey bee pollen samples (due to limited *Osmia* samples) ranging from 20 to 30 ppb concentration and simultaneously analyzed with *Osmia* pollen. Due to differing size and nature of samples, these limits may vary from sample to sample. Pesticides not included in spiked samples are listed here as QLNT (Quantification Limit Not Tested). Most of the compounds with this designation have Quantification Limits less than 5 ppb based on past 5 g bee-collected pollen samples.

active ingredient	average LOQ (ppb)	active ingredient	average LOQ (ppb)	active ingredient	average LOQ (ppb)
3-hydroxy-carbofuran	1.000	coumaphos-oxon	0.667	fenpropimorph	0.833
3-keto-carbofuran	QLNT	cyazofamid	QLNT	fenpyroximate	QLNT
acephate	30.000	cyflufenamid	2.000	fenthion	QLNT
acetochlor	0.833	cymoxanil	QLNT	fipronil	1.000
alachlor	1.000	cyproconazole	4.000	flonicamid	QLNT
aldicarb	3.333	cyproconazole	1.500	florasulam	QLNT
amitraz metab dmpf (methomyl coelutes)	13.333	diflubenzuron	1.000	fluazinam	1.000
amitraz metab dmpf	5.667	dimethenamid	QLNT	flubendiamide	QLNT
ancymidol	QLNT	dimethoate	1.000	flucarbazone	QLNT
axoxystrobin	0.500	dinotefuran	1.333	fludioxonil	1.667
azinphos-methyl	10.333	diphenamid	QLNT	flufenoxuron	QLNT
bendiocarb	1.000	diphenylamine	QLNT	flumioxazin	QLNT
bentazon	QLNT	disulfoton	QLNT	fluopicolide	QLNT
benthiavalicarb isopropyl	QLNT	dithiopyr	QLNT	fluroxypyr	QLNT
bifenazate	QLNT	diuron	1.000	flutolanil	QLNT
brodifacoum (2 isomers)	QLNT	dodine	QLNT	flutriafol	QLNT
bromacil	QLNT	epoxiconazole	QLNT	fluvalinate	QLNT
bromadiolone	QLNT	ethaboxam	QLNT	halosulfuron-methyl	QLNT
bupirimate	QLNT	ethiprole	QLNT	hexazinone	QLNT
carbofuran	0.667	etoxazole	QLNT	imazalil	0.667
chlorantraniliprole	8.333	etridiazole	QLNT	imazamox	QLNT
chlorfenvinphos	0.833	fenazaquin	QLNT	imidacloprid, 5-hydroxy	QLNT
chlorpropham	QLNT	fenbutatin oxide	QLNT	imidacloprid, olefin	QLNT
chlorsulfuron	QLNT	fenhexamid	2.333	imidacloprid, urea	QLNT
clofentezine	QLNT	fenitrothion	QLNT	imiprothrin	QLNT
coumaphos	1.667	fenpropathrin	0.500	indaziflam	QLNT

Table A2.2 Continued.

active ingredient	average LOQ (ppb)	active ingredient	average LOQ (ppb)	active ingredient	average LOQ (ppb)
kresoxim-methyl	QLNT	pinoxaden	QLNT	sethoxydim	QLNT
linuron	1.000	piperonyl butoxide	1.000	sethoxydim-sulfoxide	QLNT
malathion	1.000	pirimicarb	0.667	siduron	QLNT
mandipropamide	0.667	pirimiphos-methyl	QLNT	spinetoram 2	QLNT
mepanipyrim	QLNT	procymidone	QLNT	spinetoram I	QLNT
mesotrione	QLNT	prodiamine	QLNT	spirodiclofen	QLNT
metalaxyl	0.500	profenofos	QLNT	spirotetramat	0.500
metconazole	2.000	prometon	QLNT	spiroxamine	QLNT
methamidophos	10.000	prometryn	QLNT	sulfate (cdec)	QLNT
methiocarb	1.000	propamocarb	QLNT	sulfentrazone	QLNT
methomyl (dmpmf coelutes)	16.667	propanil	QLNT	sulfometuron-methyl	QLNT
methoxyfenozide	2.333	propargite	QLNT	tebuconazole	5.000
metolcarb	QLNT	propiconazole-1	0.667	tebupirimfos	QLNT
metrafenone	1.000	propoxur	1.500	tembotrione	QLNT
metsulfuron-methyl	QLNT	propyzamide	QLNT	thiabendazole	3.667
monocrotophos	1.000	prothioconazole	QLNT	tralkoxydim	QLNT
napropamide	QLNT	pymetrozine	QLNT	triadimenol	QLNT
oxadiazon	QLNT	pyraflufen-ethyl	QLNT	trichlorfon	1.333
oxadixyl	2.000	pyridaben	0.833	tridemorph	QLNT
oxamyl	QLNT	pyridalyl	QLNT	triflumizole	QLNT
oxyfluorfen	2.000	pyriproxyfen	QLNT	triforine	QLNT
paclobutrazol	2.000	pyroxsulam	QLNT	triticonazole	QLNT
penoxsulam	QLNT	quinoxyfen	QLNT	uniconazole	2.000
phorate	QLNT	resmethrin	QLNT	warfarin	QLNT
phosmet oxon	QLNT	rotenone	1.333		
picoxystrobin	QLNT	sec-/tert-butanol	QLNT		

Table A2.3. Summary of bee offspring responses and nest tube collection per time point. Bee offspring response at three time-points (in 2015) per site (letters). The number of male and female offspring that emerged, their weight in milligrams, the proportion of larvae that died before emergence, and the proportion female offspring produced are shown here. The average temperature per time point (in˚C) is also shown. Observations highlighted in grey were excluded from parts of the final analysis (see Methods in Chapter 2 and Appendix A2.1).

Site	Time Point	Date	Nest Tubes for Offspring Analysis	Nest Tubes for Pollen Analysis	Provisions in Pollen Homogenate	Number Female Offspring	Number Male Offspring	Proportion Female Offspring	Proportion Larval Mortality	Female Offspring Weight mg	Male Offspring Weight mg	Temp °C
A	1	20-May	4	2	18	10	5	0.67	0.11	57.28	29.93	18.23
A	2	28-May	12	2	21	36	67	0.35	0.07	58.82	31.76	23.34
A	3	3-Jun	3	2	20	7	19	0.27	0	53.4	37.18	15.98
B	1	25-May	7	2	19	19	26	0.42	0.24	54.91	35.72	13.64
B	2	29-May	19	3	23	40	66	0.38	0.19	65.41	37.1	23.24
B	3	4-Jun	7	2	13	17	16	0.52	0.18	53.67	31.06	16.89
C	1	23-May	10	2	19	50	28	0.64	0.07	55.87	34.24	13.42
C	2	30-May	3	2	23	11	14	0.44	0	59.84	30.8	21.77
C	3	5-Jun	4	2	18	16	17	0.48	0.03	56.67	32.05	14.60
D	1	21-May	18	2	22	76	78	0.49	0.08	59.64	39.16	20.63
D	2	29-May	12	3	29	28	29	0.49	0.06	58.44	34.53	17.08
D	3	4-Jun	3	2	7	11	6	0.65	0.16	63.14	30.25	15.90
E	1	21-May	1	1	8	2	2	0.5	0.2	41.55	29.71	13.91
E	2	29-May	2	3	23	13	14	0.48	0.18	55.31	27.12	22.47
E	3	4-Jun	4	2	22	7	7	0.5	0.11	48.72	29.82	16.46
F	1	27-May	2	2	16	8	5	0.62	0.2	48.37	31.56	10.13
F	2	2-Jun	10	3	35	44	46	0.49	0.09	63.13	33.4	16.50
F	3	6-Jun	6	2	17	8	23	0.26	0	61.67	30.51	15.56
G	1	25-May	7	2	23	33	26	0.56	0.02	62.87	32.48	17.60
G	2	29-May	2	1	6	12	6	0.67	0.09	58.24	40.56	12.12

Table A2.3 (Continued).

Site	Time Point	Date	Nest Tubes for Offspring Analysis	Nest Tubes for Pollen Analysis	Provisions in Pollen Homogenate	Number Female Offspring	Number Male Offspring	Proportion Female Offspring	Proportion Larval Mortality	Female Offspring Weight mg	Male Offspring Weight mg	Temp °C
G	3	4-Jun	6	2	17	19	5	0.79	0.4	56.025	29.92	23.41
H	1	23-May	8	2	19	38	32	0.54	0.1	55.02	35.73	14.72
H	2	2-Jun	11	3	23	37	54	0.41	0.07	64.33	34.29	22.65
H	3	6-Jun	3	2	19	12	12	0.5	0	66	32.12	14.70
I	1	20-May	10	2	21	47	19	0.71	0.15	60.67	28.14	15.78
I	2	24-May	14	3	34	48	49	0.49	0.09	63	31.25	22.86
I	3	28-May	4	2	10	7	30	0.19	0.18	51.62	32.37	16.35
J	1	24-May	5	2	19	28	14	0.67	0	51.67	29.15	14.54
J	2	30-May	8	3	19	8	39	0.17	0.17	56.76	29.75	19.85
J	3	5-Jun	3	2	17	3	17	0.15	0.22	55.23	27.28	22.19
K	1	23-May	3	2	18	8	4	0.67	0.31	56.56	26.98	17.42
K	2	30-May	8	3	34	22	39	0.36	0.08	54.7	28.34	23.62
K	3	5-Jun	4	2	13	11	16	0.41	0.04	55.7	26.41	16.77
L	1	20-May	17	2	18	43	40	0.52	0.14	54.53	26.88	18.27
L	2	28-May	2	2	16	0	6	0	0.74	NA	29.54	23.04
L	3	3-Jun	1	1	10	3	3	0.5	0	65.48	34.65	17.25
M	1	23-May	5	2	19	18	22	0.45	0.04	57.84	30.55	12.60
M	2	27-May	9	2	21	24	40	0.38	0.06	57.75	33.07	22.87
M	3	6-Jun	1	1	11	0	6	0	0	NA	42.15	17.80

Table A2.3 (Continued).

Site	Time Point	Date	Nest Tubes for Offspring Analysis	Nest Tubes for Pollen Analysis	Provisions in Pollen Homogenate	Number Female Offspring	Number Male Offspring	Proportion Female Offspring	Proportion Larval Mortality	Female Offspring Weight mg	Male Offspring Weight mg	Temp °C
N	1	21-May	16	2	18	65	27	0.71	0.25	64.43	39.06	16.77
N	2	29-May	23	3	31	93	82	0.53	0.04	63.78	34.52	22.57
N	3	4-Jun	5	2	19	14	25	0.36	0.14	63.72	33.49	15.39
O	1	24-May	3	2	18	17	6	0.74	0	57.93	33.56	12.22
O	2	28-May	6	2	19	23	19	0.55	0.17	63.18	29.91	22.88
O	3	3-Jun	4	2	14	1	6	0.14	0	NA	45.82	16.58
P	1	20-May	5	2	16	19	15	0.56	0.04	48.9	28.87	17.34
P	2	24-May	1	1	10	2	2	0.5	0	42.51	NA	11.61
P	3	3-Jun	10	4	35	17	16	0.52	0.31	50.25	32.28	16.27
Q	1	23-May	2	2	22	13	4	0.76	0.05	53.55	32.19	14.63
Q	2	26-May	2	2	22	12	9	0.57	0	49.59	29.16	20.84
Q	3	30-May	9	3	30	29	44	0.4	0.05	68.11	38.46	22.43

Table A2.4. Top single predictor models from model selection. The most explanatory (in terms of AIC score) single predictor models for each response variable resulting from the maximum likelihood dredge analysis. Response and predictor variables are listed with their corresponding scales and sample size number (n), which were consistent with the bee response observations numbers. For each model, we present the effect size, the estimated R^2 value based on likelihood-ratio, the degrees of freedom (df), the log-likelihood, AICc score, the delta value, and the model weight. Variables selected for the SEM analyses are highlighted in grey.

response	N	Predictor	effect size	R^2	df	log-likelihood	AICc	Δ	Weight
Pesticide Risk Predicted by Landscape Composition									
Insecticide Risk (%HQ)	51	Agriculture 2000 m	333.2	0.457	4	-293.447	595.8	0	0.639
		Shrub/wetland 250 m	-923	0.424	4	-294.952	598.8	3.01	0.142
		Open 2000 m	-852.2	0.413	4	-295.405	599.7	3.92	0.09
	50	Agriculture 2000 m	335.3	0.458	4	-288.105	585.1	0	0.643
		Shrub/wetland 250 m	-915.3	0.424	4	-289.661	588.2	3.11	0.136
		Open 2000 m	-867	0.415	4	-290.048	589	3.89	0.092
Fungicide Risk (%HQ)	51	Agriculture 500 m	0.170	0.27	61	4 68.531	-128.2	0	0.859
		Shrub/wetland 250 m	-0.764	0.2	45	4 66.127	-123.4	4.81	0.078
		Open 250 m	-0.155	0.18	55	465.524	-122.2	6.01	0.042
	48	Agriculture 500 m	0.221	0.332	4	66.035	-123.1	0	0.693
		Shrub/wetland 250 m	-1.119	0.299	4	64.846	-120.8	2.38	0.211
		Open 250 m	-0.180	0.252	4	63.291	-117.7	5.49	0.045
	46	Agriculture 500 m	0.217	0.429	4	4 65.148	-121.3	0	0.61
		Shrub/wetland 250 m	-1.112	0.407	4	4 64.264	-119.6	1.77	0.252
		Open 250 m	-0.197	0.376	4	4 63.104	-117.2	4.09	0.079

Table A2.4 (Continued).

response	N	Predictor	effect size	R^2	df	log-likelihood	AICc	Δ	Weight
Floral Diet Diversity Predicted by Landscape Composition									
Proportion Vitaceae	51	Urban 1000 m	0.874	0.180	4	-6.777	22.4	0	0.846
		Open 2000 m	2.365	0.088	4	-9.485	27.8	5.41	0.056
		Agriculture 1000 m	-0.419	0.071	4	-9.94	28.7	6.32	0.036
	50	Urban 1000 m	0.887	0.183	4	-7.023	22.9	0	0.83
		Open 1500 m	2.045	0.096	4	-9.553	28	5.06	0.066
		Forest 1750 m	-0.443	0.077	4	-10.074	29	6.1	0.039
Floral Shannon Diversity	51	Agriculture 250 m	-0.481	0.222	4	-1.061	11	0	0.476
		Shrub/land 250 m	-0.481	0.210	4	-1.435	11.7	0.75	0.327
		Urban 750 m	0.6552	0.181	4	-2.37	13.6	2.62	0.128
Proportion Rosaceae	46	Shrub/wetland 1250 m	-6.772	0.541	4	8.847	-8.7	0	0.735
		Open 250 m	-0.923	0.515	4	7.541	-6.1	2.61	0.199
		Agriculture 750 m	0.688	0.483	4	6.079	-3.2	5.54	0.046
Floral Evenness	48	Open 1500 m	1.072	0.058	4	14.798	-20.7	0	0.41
		Shrub/wetland 1750 m	1.468	0.033	4	14.16	-19.4	1.28	0.217
		Urban 500 m	0.154	0.016	4	13.73	-18.5	2.14	0.141

Table A2.4 (Continued).

response	N	Predictor	effect size	R^2	df	log-likelihood	AICc	Δ	Weight
Bee Response Predicted by Landscape Composition									
Number Female Offspring	51	Shrub/wetland 250 m	136.9	0.075	4	-222.185	453.2	0	0.468
		Forest 250 m	20.14	0.039	4	-223.169	455.2	1.97	0.175
		Agriculture 250 m	-14.03	0.035	4	-223.263	455.4	2.16	0.159
Number Male Offspring	51	Shrub/wetland 250 m	107.1	0.045	4	-223.541	456	0	0.292
		Agriculture 750 m	-21.28	0.044	4	-223.565	456	0.05	0.285
		Urban 750 m	18.48	0.0228	4	-224.13	457.1	1.18	0.162
Proportion Larval Mortality	50	Open 1000 m	-0.577	0.122	4	49.956	-91	0	0.673
		Agriculture 2000 m	0.172	0.074	4	48.624	-88.4	2.66	0.178
		Forest 2000 m	-0.112	0.039	4	47.697	-86.5	4.52	0.07
Proportion Female Offspring	51	Urban 250 m	0.256	0.019	4	-15.668	22.5	0	0.249
		Open 1500 m	-0.387	0.009	4	-15.414	22	0.51	0.193
		Agriculture 2000 m	0.113	0.009	4	-15.41	22	0.52	0.193
Female Offspring Weight	46	Open 2000 m	0.0838	0.224	4	176.801	-344.6	0	0.763
		Agriculture 750 m	-0.012	0.164	4	175.089	-341.2	3.42	0.138
		Shrub/wetland 1500 m	0.086	0.138	4	174.403	-339.8	4.8	0.069
Male Offspring Weight	48	Open 2000 m	0.028	0.052	4	197.021	-385.1	0	0.315
		Forest 2000 m	0.005	0.049	4	196.946	-385	0.15	0.292
		Agriculture 250 m	-0.002	0.018	4	196.177	-383.4	1.69	0.135

Table A2.4 (Continued).

response	N	Predictor	effect size	R^2	df	log-likelihood	AICc	Δ	Weight
Bee Response Predicted by Pesticide Risk									
Number Female Offspring	51	Insecticide Risk (HQ%)	-0.058	0.095	4	-221.636	452.1	0	0.851
		Fungicide Risk (HQ%)	-46.58	0.031	4	-223.377	455.6	3.48	0.149
Number Male Offspring	51	Insecticide Risk (HQ%)	-0.069	0.128	4	-221.218	451.3	0	0.782
		Fungicide Risk (HQ%)	-77.19	0.083	4	-222.498	453.9	2.56	0.218
Proportion Larval Mortality	50	Insecticide Risk (HQ%)	0.000	0.087	4	48.969	-89	0	0.869
		Fungicide Risk (HQ%)	15.63	0.015	4	47.08	-85.3	3.78	0.131
Proportion Female Offspring	51	Fungicide Risk (HQ%)	0.4726	0.039	4	16.73	-23.5	0	0.683
		Insecticide Risk (HQ%)	0.000	0.009	4	15.407	-21.9	1.53	0.317
Female Offspring Weight	46	Fungicide Risk (%HQ)	-0.039	0.267	4	178.116	-347.3	0	0.963
		Insecticide Risk (%HQ)	-0.000	0.156	4	174.866	-340.8	6.5	0.037
Male Offspring Weight	48	Fungicide Risk (%HQ)	-0.011	0.040	4	196.72	-384.5	0	0.706
		Insecticide Risk (%HQ)	-0.000	0.005	4	195.845	-382.8	1.75	0.294

Table A2.4 (Continued).

response	N	Predictor	effect size	R^2	df	log-likelihood	AICc	Δ	Weight
Bee Response Predicted by Diet Diversity									
Number Female Offspring	51	Floral Shannon Diversity	28.94	0.148	4	-220.09	449	0	0.796
		Floral Evenness	29.51	0.077	4	-222.142	453.2	4.1	0.102
		Floral Richness	2.514	0.023	4	-223.588	456	6.99	0.024
Number Male Offspring	51	Floral Shannon Diversity	30.6	0.187	4	-219.447	447.8	0	0.907
		Floral Richness	4.831	0.077	4	-222.679	454.2	6.4	0.036
		Floral Evenness	-16.2	0.060	4	-223.13	455.1	7.3	0.023
Proportion Larval Mortality	50	Proportion Vitaceae	-0.055	0.032	4	47.512	-86.1	0	0.253
		Proportion Rosaceae	0.0345	0.012	4	46.998	-85.1	1.03	0.152
		Proportion Rhamnaceae	0.0392	0.008	4	46.894	-84.9	1.23	0.137
Proportion Female Offspring	51	Proportion Vitaceae	-0.206	0.122	4	18.497	-28.1	0	0.651
		Proportion Rosaceae	0.145	0.058	4	16.705	-24.5	3.58	0.109
		Proportion Caprifoliaceae	0.140	0.050	4	16.501	-24.1	3.99	0.089
Female Offspring Weight	46	Proportion Rosaceae	-0.009	0.189	4	175.791	-342.6	0	0.486
		Floral Shannon Diversity	0.008	0.174	4	175.36	-341.7	0.86	0.316
		Floral Richness	0.002	0.123	4	173.985	-339	3.61	0.08
Male Offspring Weight	48	Floral Evenness	0.008	0.135	4	199.203	-389.5	0	0.628
		Floral Richness	-0.001	0.094	4	198.089	-387.2	2.23	0.206
		Proportion Caprifoliaceae	0.003	0.043	4	196.773	-384.6	4.86	0.055

Table A2.5. Summary of pesticide risk. Overall, there were 13 insecticide and 15 fungicide active ingredients detected in bee-collected pollen. Here, we show the average percent hazard quotient per time point per bee due to fungicides and insecticides for each time point at each site (letters). We also show the average expsoure per pesticide in ppb. The average across sites are shown in the 2nd row highlighted in light grey.

Site	Time Point	Fungicide Exposure (ppb)	Fungicide Risk (%HQ)	Insecticide Exposure (ppb)	Insecticide Risk (%HQ)
Average Across Sites		**110.602**	**0.041**	**107.335**	**56.463**
A	1	0.650	0.000	0.300	0.043
A	2	1.167	0.000	2.233	3.323
A	3	1.100	0.000	13.075	12.830
B	1	12.067	0.002	45.600	12.093
B	2	25.600	0.004	87.100	23.096
B	3	8.200	0.002	21.000	7.743
C	1	62.000	0.032	30.000	29.041
C	2	51.840	0.037	67.060	46.698
C	3	37.667	0.036	50.800	20.642
D	1	9.700	0.000	0.200	0.057
D	2	2.500	0.000	49.033	0.000
D	3	0.750	0.000	13.700	70.401
E	1	1064.500	0.347	221.100	536.826
E	2	267.000	0.056	82.833	392.562
E	3	46.167	0.054	60.500	225.190
F	1	0.700	0.000	0.400	0.862
F	2	0.500	0.000	0.000	0.027
F	3	0.000	0.000	11.000	0.033
G	1	590.314	0.093	785.750	0.001
G	2	78.686	0.064	453.260	258.883
G	3	76.486	0.058	343.250	189.423
H	1	0.000	0.006	13.000	0.000
H	2	0.000	0.004	0.400	15.249
H	3	0.000	0.008	0.500	13.405
I	1	151.000	0.001	1.000	0.029
I	2	61.267	0.000	430.525	20.721
I	3	67.267	0.000	329.300	3.637
J	1	48.667	0.000	0.000	0.000
J	2	54.000	0.003	59.050	49.494
J	3	53.333	0.002	57.750	36.873
K	1	2.000	0.098	0.000	58.626
K	2	15.733	0.025	400.000	34.294
K	3	19.300	0.008	298.000	49.840

Table A2.5 (Continued).

Site	Time Point	Fungicide Exposure (ppb)	Fungicide Risk (%HQ)	Insecticide Exposure (ppb)	Insecticide Risk (%HQ)
Average Across Sites		**110.602**	**0.041**	**107.335**	**56.463**
L	1	906.500	0.192	0.000	0.000
L	2	79.420	0.013	102.500	62.571
L	3	57.960	0.009	95.600	25.360
M	1	2.700	0.000	0.400	0.057
M	2	42.333	0.012	13.150	2.236
M	3	67.475	0.066	159.133	50.712
N	1	0.000	0.000	0.600	0.086
N	2	0.000	0.000	0.300	0.043
N	3	0.000	0.000	0.700	0.100
O	1	393.967	0.225	158.050	81.591
O	2	71.914	0.031	203.300	204.732
O	3	13.317	0.006	118.275	80.936
P	1	594.175	0.244	1.400	13.142
P	2	246.567	0.146	276.633	133.606
P	3	354.200	0.186	414.150	109.814
Q	1	0.000	0.000	0.200	0.029
Q	2	0.000	0.000	1.150	1.526
Q	3	0.000	0.000	0.800	1.111

Table A2.6. Assessment of robustness of results. We replaced all landscape variables in the final number of female offspring and female offspring weight paths with the two most adjacent scales from 250 to 2000 m. Here, we show the mean (per path) absolute value of change, between the highest and lowest scales tested, in both effect size and p-value estimates. We also show the maximum absolute value change for both effect size estimates and p-values from each path. We found two differences in significance across all links, both of which were in the female weight path. There were no changes in the direction of effects across scales.

Bee Response	Original Landscape Variable	Other Scales Tested	Maximum Correlation Coefficient Change	Mean Correlation Coefficient Change	Maximum p-value Change	Mean p-value Change	Changes in Significance of Hypothesized Relationships	Scales with Changes
Number of Female Offspring	Agriculture 2000 m	1750 m, 1500 m	0.011	0.002	0.013	0.001	none	
	Agriculture 250 m	500 m, 750 m	0.039	0.005	0.070	0.010	none	
Female Offspring Weight	Open 2000 m	1750 m, 1500 m	0.050	0.013	0.025	0.010	the negative effect of Fungicide Risk on Female Offspring Weight (p=0.052) becomes 95% significant (p<0.05)	1750 m, 1500 m
	Agriculture 500 m	250 m, 750 m	0.073	0.008	0.016	0.003	none	
	Shrub/wetland 1250 m	1000 m, 1500 m	0.088	0.009	0.042	0.005	the negative effect of Shrub /wetland on proportion Rosaceae (p=0.011) becomes less significant (p=0.058)	1500 m

APPENDIX 3: SUPPLEMENTARY MATERIALS FOR CHAPTER 3

Appendix A3.1. Detailed pesticide quantification methods for *Apis*.

Sample preparation

Pollen samples were extracted by a modified QuEChERS method and screened for 49 pesticides by liquid chromatography mass spectrometry (LC-MS/ MS).

Three grams of pollen were extracted with 14 mL of acetonitrile/water (1:1, v/v) and homogenized using 10 g of ceramic beads and a Bead Ruptor 24 [OMNI International, USA]. After complete homogenization, 6.5 g of EN 15662 salts were added (4 g MgSO4; 1 g NaCl; 1 g sodium citrate tribasic dihydrate; 0.5 g sodium citrate dibasic sesquihydrate). Samples were then shaken and centrifuged at 7300 × g for 5 minutes. After centrifugation, 1000 µl of supernatant was collected and transferred into a d-SPE (dispersive solid phase extraction) tube containing 150 mg PSA, 900 mg MgSO4. After the d-SPE step, 490 µL of sample was collected and 10 µL of internal standard solution (d_4-imidacloprid 0.6 ng/µL; d_5-pyrimethanil 0.6 ng/µL, d_3-thiametoxam 0.6 ng/µL, d_3-propiconazole 2.5 ng/µL, d_3-amitraz 0.06 ng/µL) was added. Samples were filtered (0.22 µm, PTFE) and analyzed immediately by LC-ESI-MS/MS.

Liquid Chromatography and Mass Spectrometry

Sample analysis was carried out with a Vanquish Flex UHPLC system [Dionex Softron GmbH, Germering, Germany] coupled with a TSQ Quantis mass spectrometer [Thermo Scientific, San Jose, CA]. The UHPLC was equipped with a Kinetex EVO C18 column (150 mm × 2.1 mm, 2.6 µm particle size). The mobile phase consisted of (A) Water with 5 mM ammonium formate and 0.05% formic acid and (B) Acetonitrile/Water (9:1, v/v) with 5 mM ammonium formate and 0.05% formic acid. The temperature of the column was maintained at 40°C throughout the run and the flow rate was set at 500 µL/min. The elution program was the following: 2 min equilibration (1% B) prior to injection, 0-0.5 min (1% B, isocratic),

0.5-1.5 min (1%-50% B, non-linear gradient, curve 3), 1.5-9 min (50%100% B, non-linear gradient, curve 6), 9-10.8 min (100% B, column wash), 10.8-11 min (100%-1% B, linear gradient), 11-12 min (1% B, re-equilibration). The flow from the LC was directed to the mass spectrometer through a Heated Electrospray probe (H-ESI). The settings of the H-ESI were: spray voltage 4000 V, Sheath gas 60 (arbitrary unit), Auxiliary gas 20 (arbitrary unit), Sweep gas 1.5 (arbitrary unit), Ion transfer tube temperature 325°C, Vaporizer temperature 350°C.

The MS/MS detection was carried out using the Selected Reaction Monitoring (SRM) mode. Two transitions were monitored for each compound: one for quantification and the other for confirmation. The resolution of both Q1 and Q3 was set at 0.7 FWHM, the cycle time was 0.8 s and the pressure of the collision gas (argon) was set at 1.5 mTorr.

Analyte Calibration and Quantification

For each analyte, the two most abundant transitions were monitored. The most abundant one was used for quantification and the other was used for confirmation. Calibration solutions were prepared in extracted pesticide-free matrix in the range of 0.00001−1 ng/µl. Analyte concentrations in the final extracts were determined based on the fitted curves and back-calculated for a concentration taking extraction losses into consideration (as in McArt et al., 2017).

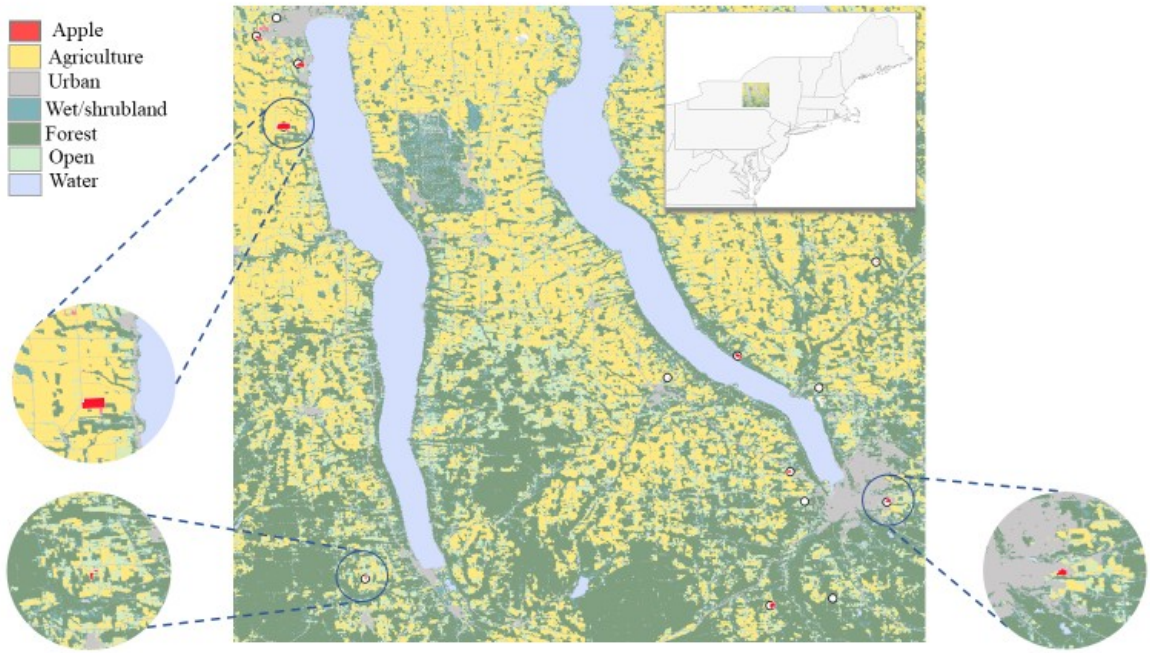

Figure A3.1. The 14 sites (white dots) used in our study were located in the Finger-lakes region of upstate New York (see cut-out top right). Ground-truthed (see Methods) apple orchards (bright red polygons) and apple land cover (muted red) are shown here. To show the diversity of our sites, we show the proportion agriculture, urban area, wet and shrubland, forest, open area, and open water estimated by the Crop Scape Data Layer. Land cover and apple orchard polygons within a 3 km radius surrounding sites are shown here for three of the sites.

Table A3.1. Sampling dates at each site for both species. The number of hives (*Apis*) and nest tubes (*Osmia*) from which beebread and pollen provision homogenates were created are shown here, along with the dates for which pesticide data are missing. Apple bloom start date and end date are listed for each site, except for sites where bloom began prior to our first sampling date, which are denoted as "prior".

Species	Site	Bloom Start	Bloom End	Sampling Date	# Dates	# Hives	Missing Pesticide Data
Apis	AB	Prior	5/20	5/18	1	4	
Apis	BD	Prior	5/30	6/5	1	3	
Apis	HG	Prior	5/23	5/19	1	4	
Apis	HS	5/11	5/25	5/18	1	3	
Apis	IC	Prior	5/30	5/18	1	3	
Apis	IT	Prior	5/24	5/19	1	3	
Apis	KI	Prior	5/30	6/5	1	2	6/5
Apis	LA	Prior	5/23	5/15	1	3	
Apis	LF	Prior	5/28	5/18, 6/2	2	4, 3	
Apis	LT	Prior	5/23	5/19	1	3	
Apis	OK	Prior	5/29	5/15, 6/3	2	3, 3	5/15
Apis	RE	5/11	5/28	5/18	1	4	
Apis	RF	Prior	5/28	5/18, 6/1	2	4, 4	
Apis	WH	Prior	5/23	5/18	1	4	
Species	**Site**	**Bloom Start**	**Bloom End**	**Sampling Date**	**# Dates**	**# Nest Tubes**	**Missing Data**
Osmia	AB	Prior	5/20	5/20, 5/28	2	2, 2	
Osmia	BD	Prior	5/30	5/30, 6/5	2	2, 2	
Osmia	HG	Prior	5/23	5/23	1	2	
Osmia	HS	5/11	5/25	5/20, 5/24	2	2, 1	
Osmia	IC	Prior	5/30	5/24	1	2	
Osmia	IT	Prior	5/24	5/21	1	2	
Osmia	KI	Prior	5/30	5/30	1	2	
Osmia	LA	Prior	5/23	5/21	1	2	
Osmia	LF	Prior	5/28	5/20, 5/28	2	2, 2	
Osmia	LT	Prior	5/23	5/23	1	2	
Osmia	OK	Prior	5/29	5/21, 5/29, 6/4	3	2, 3, 2	
Osmia	RE	5/11	5/28	5/24	1	2	
Osmia	RF	Prior	5/28	5/20, 5/24, 6/3	3	2, 1, 4	
Osmia	WH	Prior	5/23	5/23	1	3	

Table A3.2. The 14 active ingredients that were tested but *not* found above the limit of quantification (LOQ) in both *Apis* beebread and *Osmia* pollen provisions. Average LOQ per site per species is shown here for each active ingredient. For information on the 19 active ingredients that *were* detected above the LOQ, see Table 3.1 in Chapter 3. For *Omsia*, LOQ estimates are based on pesticides spiked into 5 g honey bee pollen samples (due to limited *Osmia* samples) ranging from 20 to 30 ppb concentration and simultaneously analyzed with *Osmia* pollen. Due to differing size and nature of samples, these limits may vary between samples. Pesticides not included in spiked samples are listed here as QNLT (Quantitation Limit Not Tested). Most of the compounds with this designation have Quantitation Limits less than 5 ppb based on past 5 g bee-collected pollen samples. For *Apis*, LOQ values were calculated at three times the limit of detection, which corresponded to the concentration that gives a signal-to-noise ratio of 3, based on 3 g of clean honey bee pollen spiked with pesticides at concentrations ranging from 0.01 ppb to 1000 ppb.

active ingredient	***Apis***	***Osmia***
amitraz	0.04	5.67
azoxystyrobin	0.14	0.50
boscalid	70.00	1.17
chlorantraniprole	0.70	8.33
coumpahos	35.00	1.67
cyflufenamid	11.20	2.00
dithiopyr	350.00	QLNT
fluopicolide	7.00	QLNT
metalaxyl	0.11	0.50
propamocarb	0.11	QLNT
propiconazole	1.40	0.67
quinoxyfen	0.70	QLNT
spinetoram_j	0.14	QLNT
triflumizole	0.11	QLNT

Table A3.3. Apple land cover radius selection. We used single-predictor models to choose the most predictive apple land cover radius for the response proportion *Malus* pollen (log plus 0.01 transformed) for both species. AIC scores for all 6 models for each species are shown here. The lowest-ranking radius (marked in grey) for *Apis* was 3 km and for *Osmia* was 1 km, used in our analysis as the "*Malus* foraging radius" for each species.

Response	Transformation	Predictor	Radius	AIC-score *Apis*	AIC-score *Osmia*
proportion *Malus* pollen	log+0.01	Proportion Apple Land Cover	3 km	38.55	55.34
proportion *Malus* pollen	log+0.01	Proportion Apple Land Cover	2 km	39	55.09
proportion *Malus* pollen	log+0.01	Proportion Apple Land Cover	1.5 km	39.41	54.32
proportion *Malus* pollen	log+0.01	Proportion Apple Land Cover	1 km	40.53	53.92
proportion *Malus* pollen	log+0.01	Proportion Apple Land Cover	500 m	40.81	55.65
proportion *Malus* pollen	log+0.01	Proportion Apple Land Cover	250 m	40.71	58.41